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Samson Wright's Applied Physiology

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Professor Samson Wright had begun preliminary work on the 10th Edition of his *Applied Physiology* before his death in March 1956. At first the new editors thought that they might produce a new edition based very largely on the text of the 9th Edition published in 1952. However, the tremendous advances which have taken place in physiology since that time soon made them realize that such a course was quite impracticable and they decided that a much more drastic revision was necessary if the 10th Edition was to catch the spirit of Samson Wright's whole approach to the subject. They have therefore made radical changes in nearly all sections of the book.

Firstly, they have added a new section on the Special Senses, the Ear and the Eye. This fills a gap which has been criticized in the past. Secondly they have entirely rewritten the sections on the Kidney and Body Water, Heart and Circulation, Respiration, and Muscle and the Nervous System; and thirdly, they have extensively revised the sections on Blood, Endocrine Glands, Metabolism, and Nutrition.

In producing this 10th Edition of *Applied Physiology* they are fully aware of the difficulties involved in trying to follow in Samson Wright's footsteps, but they have throughout attempted to fulfil his original aim, to present physiology as a basis for the understanding of medicine.

Professor Cyril Keele was a student of Samson Wright from 1924 and, after qualification and five years of clinical work, he was so stimulated and attracted by his exposition of physiology that he joined his staff in 1933 and was a member of his department until he helped him to establish a separate department of pharmacology in 1938. He continued to teach certain aspects of physiology, including clinical demonstrations, for many years after this, and this explains how a pharmacologist comes to be associated with what it is hoped and believed will be a fruitful partnership. Professor Eric Neil worked with Samson Wright as Reader in Physiology in the University of London for a number of years and succeeded him as John Astor Professor of Physiology on his death.

The new format of the book with its large double-column pages has been introduced primarily for economic reasons and those who dislike double columns should realise that although production costs have increased by nearly fifty per cent. since the 9th Edition was published the price of this new edition has increased by only eight-and-a-half per cent. It is hoped that the much clearer type face used, the more prominent and more logically arranged headings, the elimination of footnotes, and the concentration of references at the end of each section will make this new edition much easier to read than its predecessors.

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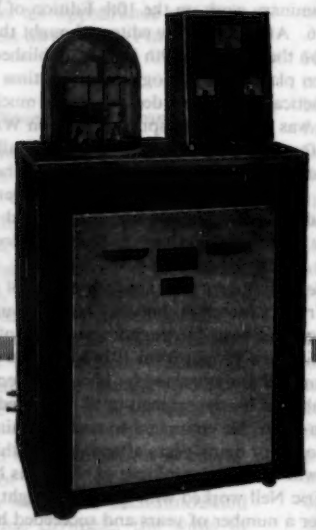

The photograph above shows an evaporated carbon replica of filter paper fibres, shadow-cast with palladium-gold alloy at 45° and photographed in the electron microscope. Magnification 57000 ×. The specimen was prepared on a "Speedivac" 12E6 coating unit. (Photograph by courtesy of Aeon Laboratories.)

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7. The Endotheliochorial Placental Membrane of Carnivora

In this type of membrane a lamina of trophoblast separates the maternal blood vessels, lined by endothelium, from the endothelium of the foetal capillaries located in the subjacent stroma. An endotheliochorial placental relationship of this kind occurs typically in the cat (Grosser, 1927; Wislocki & Dempsey, 1946a; Amoroso, 1952; Dempsey & Wislocki, 1956) and, with the exception of the hyena, *Crocuta crocuta*, (Amoroso, 1955a, 1959a, 1959b) might be regarded as characteristic of Carnivora in general.

a. Topography of the Placental Lamellae

Basically, the placental lamellae consist of dark-staining, trophoblastic plates which run more or less vertically from the placental face, and which are separated by light-staining bands of foetal connective tissue in which the slender foetal capillaries are located (Plate III: L). In the long axis of the lamellae, surrounded by trophoblast, the tortuous sinusoidal, maternal capillaries are lodged, and these are lined by an endothelium which is especially thick. The trophoblast, which consists of an outer syncytial and an inner cellular layer, also encloses pale-staining, decidual giant cells, lodged between the maternal blood vessels (Plate III: L and Plate IV: T). Both the giant cells and the maternal capillaries are invested with a layer of amorphous material, of variable thickness.

b. Cytological Specializations of the Maternal Elements of the Lamellae

Because of their characteristic topography, the various parts of the placental membrane are easily recognized in thin sections. As observed with the electron microscope (Plate IV: T, U), the endothelial cells of the maternal capillaries are characteristically enlarged and their cytoplasm has a spongy appearance, owing to the presence of numerous irregular vacuoles containing a flocculent precipitate, which resembles maternal blood plasma. Between the vacuoles lies the formed cytoplasm of the cells which contain, in addition to swollen mitochondria and the Golgi complex, many of Palade's microgranules.

In some instances, the vacuoles may communicate directly with the lumen of the capillary and it seems possible that they may represent invaginations of the surface plasma membrane, whereby blood plasma is transferred into the cell (Dempsey & Wislocki, 1956). It would thus appear that the absorptive surface of the cells is organized in a fashion comparable to that described in the yolk-sac epithelium of the guinea-pig, where, it has been suggested, absorption vacuoles segregate uterine milk in the interior of the cells by means of similar microlabyrinthine canals (Plate IV: V, W; Dempsey, 1953).

Palade (1956) has shown that in many cells the membrane is infolded extensively and that such folds may carry the cell membrane to positions deep in the cell. Moreover, he has demonstrated that such deep folds often appear to be close to isolated vesicles in the cytoplasm and that such vesicles are often arranged so as to suggest that they might have formed from the pinching-off of a recessed tip of such a fold, or that they might represent vesicles destined to coalesce with it.

This membrane vesiculation mechanism has a number of interesting features. First, it provides a means whereby particles can move from one side of a membrane to the other without going through it. Second, it makes it unnecessary to postulate any holes or pores in a membrane in order to explain the passage of particles from one side of a membrane to the other. Third, specificity of binding groups on the membrane would permit great selectivity in transfer operations. Finally, it may be surmised that membrane vesiculation might be a feature of certain ion-pump mechanisms. The synaptic vesicles discovered by De Robertis & Bennett (1954, 1955), and by Palade (1954) and Palay (1954), might be manifestations of such a process.

The perivascular basement membrane of the maternal capillaries of the cat's placenta varies a good deal in thickness and may even be absent, so that in certain locations the plasma membrane of the endothelial cells and that of the syncytial trophoblast are directly contiguous (Plate IV: U). The rather smooth and regular apposition of the basement membrane with the endothelial plasma membrane, in contrast to its scalloped and irregular contour adjacent to the trophoblast (Plate IV: U), suggests that it is being constantly eroded and absorbed by the trophoblast and renewed by the endothelium. In this connexion it might be pointed out that Dempsey & Wislocki (1956) have suggested that it may be the secretion of this material which requires some of the cytological specializations alluded to above.

An amorphous substance continuous with, and in every respect similar to, the basement membrane surrounding the maternal capillaries separates the decidual giant cells from the trophoblast (Plate IV: T). The cytoplasm of the giant cells is foamy because of innumerable rather small vacuoles, the appearance of which under the electron microscope agrees with the concept that the amorphous connective tissue forming the ground substance may in part be derived from these decidual cells.

c. Cytological Specializations of the Foetal Elements of the Lamellae

The structural characteristics of the syncytial trophoblast, as seen with the electron microscope, are illustrated in Plate IV: T, U. Its most noteworthy feature pertains to the uneven character of its limiting plasma membranes. The irregular contour of the syncytial margin which faces the perivascular membrane supports the suggestion that it is engaged in erosive activities in much the same way as the tongues of chorionic tissue in the junctional zone of the cat's placenta (Plate III: S). In this zone, the glandular epithelium adjacent to the chorion becomes converted into symplasmic masses which disintegrate and are variously phagocytosed and absorbed (Wislocki & Dempsey, 1946a; Amoroso, 1952, 1955a). This suggestion, that the syncytium erodes and digests the perivascular basement membranes, was first advanced by Dempsey & Wislocki (1956), and extends a known action of the trophoblast to a later time and a different site from those which have been previously described. (See also Wislocki & Dempsey, 1946a; Amoroso, 1952.)

Another feature of the syncytial trophoblast pertains to the specialization of its surface plasma membrane which adjoins the foetal stroma and capillaries. Along this margin, in the gaps between the cellular trophoblast or where the cytotrophoblast disappears in the course of gestation, the syncytium extends branched, foot-like contacts down to the smooth basement membrane which separates the chorion from the foetal stroma (Plate IV: U). These pseudopodia are surrounded by anastomosing extracellular spaces and resemble

the podocytes exhibited by the visceral epithelium of Bowman's capsule (Hall, Roth & Johnson, 1953; Rinehart, Farquhar, Jung & Abul-Haj, 1953; Dempsey & Wislocki, 1955). The suggestion has been made that the extracellular compartments are concerned in active fluid transfer and that the direction of flow is here predominantly from the foetal to the maternal tissues (Dempsey & Wislocki, 1956).

In contrast to the syncytium, the cytotrophoblastic cells—the number of which diminishes as pregnancy advances—possess large vesicular nuclei, are quite smooth and even, and do not exhibit the foot-like contacts and extracellular spaces displayed at the interface between the syncytium and the basement membrane. In the regions where a foetal capillary closely approaches or even indents the trophoblast, the two basement membranes remain distinct and do not fuse.

8. The Haemochorial Placental Membrane of Rodents

The chorio-allantoic placenta of Myomorpha (rats, mice), like that of the Lagomorpha (rabbits) and Hystricomorpha (guinea-pigs) is labyrinthine and in its finer structure haemochorial, in Grosser's terminology. This type of membrane represents the maximum expression of the invasive activity of the trophoblast. When observed with the light microscope at the end of gestation, the placenta of the rat or the rabbit is seen to consist of exceedingly thin laminae of epithelial cytoplasm separating the foetal capillaries from the maternal blood, and the difficulty of establishing the nature of the separating membrane is at once apparent (Plate III: M).

In the past, investigators of the placenta of the rat and of the rabbit have dissented widely in their interpretations of the nature of the epithelial cytoplasm forming the confining walls of the maternal blood channels. As observed with the light microscope, the chorion is ordinarily described as being composed initially of cytotrophoblast which later becomes transformed into syncytial trophoblast. This layer of syncytium, which was believed to vary considerably in depth, has been generally considered to be separated from the foetal blood vessels by wisps of argyrophil collagenous fibres (Wislocki, Deane & Dempsey, 1946; Amoroso, 1952). Eventually, however, the reticular fibres decrease in number, so that at term only the thinnest plates of syncytial cytoplasm intervene between many of the foetal capillaries and the maternal blood.

The various elements of the placental barrier of the rat during late gestation are illustrated in Plate V: X–Z. The most noteworthy feature of these electron micrographs is that the separating membrane is composed exclusively of foetal elements. At the stages represented, the barrier between the two blood streams consists typically of two or three sheets of cellular trophoblast, with the innermost cells resting upon a basement membrane which intervenes between the chorionic epithelium and the foetal capillaries. The outermost trophoblastic cells form the confining walls of the maternal blood channels, and their surfaces possess irregularities of different sizes and shapes which project into the blood lacunae.

The walls of the foetal capillaries adjacent to the trophoblast possess a basement membrane of their own, and the two contiguous basement membranes are everywhere separated by thin strands of collagen (Plate V: Y). It would thus appear that, in its fine structure, the rat's placenta is haemochorial and not haemo-endothelial in character (as Mossman (1926) and others supposed), its labyrinth possessing throughout gestation a complete layer of trophoblast which, in addition, is cellular and not syncytial as was once believed.

Electron micrographs of the rabbit's placenta reveal that the labyrinth is also haemochorial and not haemo-endothelial, and that the placental membrane consists also of imbricated sheets of trophoblast cells (Wislocki & Dempsey, 1955a).

9. The Anthropoid Haemochorial Membrane

The findings in the labyrinth of the rodent's placenta, as well as in that of the rabbit's, are very different from those found in human placental villi, where the trophoblast, which constitutes the principal component of the placental membrane, consists of an inner cellular layer (the Langhans' cells) adjacent to the foetal stroma, and an outer syncytial layer (the syncytium) which is bathed by the circulating maternal blood in the intervillous space.

In electron micrographs, the Langhans' cells stand out conspicuously (Plate V: BB). Both their cytoplasm and large nuclei are less dense than those of the syncytium. The Langhans' cells are apposed on their inner or basal surfaces to the basement membrane of the chorionic villi and on their outer surfaces to the syncytium; however, occasionally, small tongues of cytotrophoblast, plentifully supplied with microvilli, protrude into the intervillous space. Laterally, many of the Langhans' cells abut one another, but cell boundaries are often inconspicuous.

Wislocki & Bennett (1943) maintained that some residual Langhans' cells appeared to be present in the chorionic villi at term, and examination of the placental villi at full term with the electron microscope adds substantial proof of their conclusion, by demonstrating numerous, flattened, basally located Langhans' cells, distinguishable from the syncytium by their clearer cytoplasm.

In contrast to the Langhans' cells, the syncytium contains small, irregularly shaped, ovoid nuclei which exhibit considerable electron density. The syncytial cytoplasm has a foamy, finely granular appearance, owing to the presence of numerous, round or oval, clear vesicles of rather uniform size and granular outline (Plate V: BB, CC). The vesicles of the syncytium which have this beaded appearance are believed to be equivalent to the cytoplasmic basophilia observed with the light microscope (Wislocki & Dempsey, 1948), which Dempsey & Wislocki (1945) have shown to be attributable to ribonucleoprotein. Wislocki, Dempsey & Fawcett (1948) have postulated that the basophilic substance of the syncytium synthesizes the foetal plasma proteins until such time as the foetal liver takes over that function. This suggestion is in keeping with the steady decline of the cytoplasmic basophilia of the syncytium as pregnancy advances.

Also present in some regions, in a zone just beneath the microvilli, there are variable numbers of quite large vacuoles surrounded by indistinct membranes (Plate V: CC). These large vesicles are encountered most frequently in the marginal cytoplasmic zone of the syncytium and are believed to be formed as the result of absorption of maternal plasma by pinocytosis (Wislocki & Dempsey, 1955b).

In the later stages of pregnancy, the syncytium forms an attenuated membrane at many places over the surface of the chorionic villi. But thin as these areas may appear to be, they are cytologically very complex and are revealed by electron microscopy, as well as by light microscopy, to consist of a

thin lamina of syncytial cytoplasm which rests on a thick basement membrane, a connective-tissue space containing collagen fibres, and the wall of a dilated capillary composed of an additional basement membrane lined internally by endothelium. An interesting feature of the epithelial cytoplasm of the placental membrane is that, no matter how thin these plates become, a layer of cellular trophoblast (Plate V: AA) is demonstrable at all times in the chorionic villi (Wislocki & Dempsey, 1955b).

Bremer (1916), it will be recalled, likened the thinned-out "epithelial plates" overlying the foetal capillaries to the glomerular epithelium of Bowman's capsule, and regarded them as inert areas through which the foetal waste products are excreted. Electron microscopy reveals, however, that there is actually very little structural similarity between these various membranes. Hence, Bremer's postulate must now be regarded as having no convincing morphological basis.

The plasma membrane and basal cytoplasm of the syncytium, apposed to the delicate plasma membranes of the Langhans' cells, are quite dense in character and often dip between the latter cells to abut directly upon the basement membrane. However, the most noteworthy feature of the syncytial trophoblast concerns the irregular character of its plasma membrane bordering the intervillous space. Along this margin, the syncytium is beset with a profusion of microvilli of varying appearance (Plate V: AA–CC). Some are long and slender, others are shorter and thicker, while others are quite flat and sparse.

The presence of long slender microvilli on the surface of the syncytium is a feature which was unsuspected on the basis of light microscopy. On the other hand, the occurrence of short, irregular, stub-like microvillous projections on the surface of the chorionic villi at term is in keeping with the presence of the "striated" or "brush" border, first described by Minot (1889) and since fully documented by Wislocki and his associates.

The structural characteristics of cells engaged permanently in absorptive activities seem to depend on the nature of the surface plasma membranes, the disposition of mitochondria and sometimes the occurrence of absorption vacuoles (Dempsey, 1953; Palade, 1953). Consequently, the microvilli on the free surface of the syncytium might be regarded as a device for increasing the surface area for absorption. On the other hand, the microvilli on the surfaces of cells not ordinarily concerned with absorption (e.g., decidual gland cells) would presumably be involved in the process of secretion. It is clear, therefore, that "microvilli do not necessarily bespeak either the one process or the other; instead, they would appear, as the case may be, to increase either the effective absorbing or secretory surface" (Wislocki & Dempsey, 1955b). Microvilli must, therefore, await further study and assessment before their entire significance is established.

New details of the structure of the placenta have been revealed by the electron microscope, but any fresh conclusions that may be drawn must be tested further.

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PLATE I, FIGS. A—F

FIG. A. An early stage in the development of the yolk-sac placenta in the rabbit (about 8 days after insemination), showing details of the vascular trilaminar omphalopleur (chorio-vitelline placenta); e.d.: endoderm; v.: vitelline vessels; t.r.: trophoblast; d.u.: degenerating uterine epithelium and symplasma.

(magnification × 350)

FIGS. B, D. Photomicrographs showing the development of the placental membrane in the sow at two stages of gestation. At 32 days after insemination (fig. B), the chorion consists of low folds which are matched by corresponding folds of the endometrium. The foetal epithelium (f.e.) is uniformly columnar and light-staining, whereas the uterine epithelium (u.e.) is cuboidal and intensely basophilic. By mid-pregnancy (fig. D) the folding of the chorion has become more complex, and chorionic fossae (c.f.) and chorionic ridges (c.r.) are now quite distinct; in the depths of the fossae the cells remain tall and columnar, whilst on the sides and over the summits of the ridges they become cuboidal, flat, or even syncytial, and are penetrated by capillaries.

(fig. B, magnification × 275)
(fig. D, magnification × 125)

FIG. C. Section of a portion of the embryonic swelling of a cat about 19 days pregnant, showing how the vascularized mesoderm of the allantois (Al.) reaches and makes contact over a broad zone with the non-vascular extra-embryonic somatopleur of the chorionic placenta to form the chorio-allantoic membrane.

(magnification × 60)

FIG. E. Photograph of the allantoic surface of the chorio-allantoic membrane, showing vesicles or cysts of various sizes and their relation to the allantoic vessels. These vesicles appear on the chorio-allantoic membrane, where there is no fusion of the endometrium and the chorion. They may arise as enlargements of the intervillous fossae or by the expansion of a space between the bases of the villi. Heuser (1927) believes that these structures are "secretory in function, yielding digestive ferments to act upon the uterine milk".

FIG. F. Photomicrograph through an irregular areola and the adjacent parts of the inter-areolar regions (i.a.). Note the differences in the topography of the foetal membranes, as well as the differing character of both the foetal and maternal epithelia in these two areas. The dome-like covering of trophoblast (t.r.) shows little tendency to form folds, whereas the uterine wall associated with it is complicated by ridges and papilla-like structures (u.p.). The areolae are believed to be associated with the absorption of uterine milk (u.m.).

(magnification × 75)

PLATE II, FIGS. G—K

FIG. G. Electron micrograph through a region of the chorionic ridge of a pig's placenta in the last third of pregnancy. A chorionic cell (top of figure) is seen covering several uterine epithelial cells (base of figure); the line of cellular apposition of the maternal and foetal epithelia runs irregularly across, and the interdigitating microvillous processes are discernible. An intra-epithelial foetal capillary (f.c.) with contained erythrocytes, and a limiting basement membrane (b.m.) may be seen at the top centre. Beneath it, spaces of vacuoles without definable membranes appear in the chorionic epithelium. At lower right corner a small part of the uterine basement membrane is visible. Note the clear apical zone of the uterine epithelium.

(magnification × 5,000)

FIG. H. Electron micrograph of section through cells from the chorionic fossa. Numerous colloid droplets are seen in the bases of the epithelial cells. Also located basally are strands of ergastoplasm which in some areas are pinched off to form tiny vesicles similar in size to the smallest colloid droplets. The chorionic basement membrane upon which the epithelium rests can be seen at bottom of figure.

(magnification × 5,000)

FIG. J. Electron micrograph of foetal–maternal junction from the region of a chorionic ridge. The chorionic cells are at the top and the uterine epithelium at the base of the figure. The extensive interdigitations of the short blunt microvilli which extend from the plasma membranes of both types of cells are apparent. Near the apical portion of the trophoblastic cells are a goodly number of mitochondria. In contrast, the cytoplasm of the endometrial epithelial cells apposed to the chorionic ridges possesses a relatively pale apical zone beneath the surface membrane devoid of mitochondria.

(magnification × 8,000)

FIG. K. The zone of apposition of foetal and maternal epithelial cells in the chorionic epithelium of the mare's placenta. At the apical surfaces of the chorionic cells (top), the plasma membrane is closely apposed to the cell membrane of the endometrial epithelium (bottom) in a complex fashion. This complicated dovetailing of the cell membranes is quite different from that of the pig's placenta, and more nearly resembles that of the cow (figs. N and P). The apical cytoplasm of the endometrial cells apposed to the chorion differs in certain respects from that of the uterine epithelial cells in the pig's placenta. Instead of a relatively pale zone near the junction with the chorion a goodly number of mitochondria are present. Compare with fig. N.

(magnification × 1,000)

PLATE III, FIGS. L—S

FIG. L. Details of the lamellae from the placental labyrinth of a cat at about the 30th day of pregnancy, as seen with the light microscope. Observe the dark-staining syncytial trophoblast (s.t.) which encloses decidual giant cells (g.c.) and maternal capillaries (m.c.). Notice the pale-staining matrix which accompanies the maternal blood vessels and giant cells and intervenes between them and the syncytial trophoblast. Observe the hypertrophied endothelial cells lining the maternal capillaries and the thin-walled foetal blood vessel (f.c.) lying in the chorio-allantoic mesenchyme (a.c.m.) adjacent to light-staining cytotrophoblast (c.t.).

(magnification × 225)

FIG. M. Trophoblastic tubes from the placental labyrinth of a rabbit at about the 17th day of pregnancy, for comparison with fig. L. The centre of the photomicrograph shows foetal capillaries containing large nucleated red corpuscles, and about them may be seen two anastomosing trophoblastic tubes containing smaller, maternal erythrocytes. The foetal blood vessels are closely apposed to the walls of the trophoblastic tubes, which consist of cellular trophoblast and not syncytial trophoblast, as has been heretofore believed. Except for the fact that the maternal blood directly bathes the trophoblast and is not contained in vessels lined by endothelium, the resemblance of the trophoblastic tubules of the rabbit to the lamellae of the placental labyrinth of the cat is very striking indeed.

(magnification × 225)

FIG. N. Electron micrograph of the foetal–maternal junction in the bovine placentome. One large trophoblastic cell (top) is seen covering several cryptal cells (bottom). The contact surface is characterized by a great number of microvilli from both sides interdigitating with each other. In the trophoblastic cell an accumulation of mitochondria is seen in the zone adjacent to the microvilli.

(magnification × 10,000)

FIG. P. Electron micrograph of bovine trophoblastic and cryptal cells with microvilli. To the upper left, a trophoblastic giant cell is seen. The large nucleus contains two distinct nucleoli which show a reticular pattern. This is also the case in the nucleolus of the ordinary trophoblastic cell (upper right).

(magnification × 10,000)

FIG. Q. Portions of two foetal villi and the intervening intercrypt column of a red deer (6-cm. foetus), showing the nature of the epithelial investment of the maternal septum (m.s.). At upper left corner of figure, the trophoblast (t.r.) and the covering epithelium of the maternal septum are closely apposed, creating an epitheliochorial relationship. At lower part of figure, the two epithelial layers (maternal uppermost) have been artificially separated and each contains a variable number of binucleate giant cells (g.c.). Foetal (f.c.) and maternal (m.c.) capillaries lie in the allantoic and maternal mesenchyma respectively.

(magnification × 175)

FIG. R. Electron micrograph of bovine cryptal cells with an extension of a process from a trophoblastic cell between them. Microvilli are also found here. In the infra-nuclear zone of the cells, large osmiophilic granules are seen surrounded by intra-cytoplasmic membranes. At bottom of picture the basement membrane is seen.

(magnification × 8,000)

FIG. S. Photomicrograph of junctional zone (j.z.) and deep glandular zone (g.z.) of the cat's placenta at mid-gestation. Here the enlarged terminal ends of the primary lamellae (the cytotrophoblastic arcades (c.a.)) of the labyrinth are followed by an irregular junctional or transition zone, consisting of maternal cell debris, and a zone of glandular symplasma. The figure illustrates very sharply three syncytial sprouts (s.s.) extending from the trophoblastic arcades (c.a.) and entering the glandular zone (g.z.). In doing so, the syncytium gradually erodes and destroys the maternal tissues. The very dark pycnotic masses, at the bottom of the figure, are fused masses of uterine epithelial cells (symplasma) derived from disintegrating uterine crypts.

(magnification × 140)

PLATE IV, FIGS. T—W

FIG. T. Electron micrograph through a typical placental lamella from a cat during mid-gestation. The section illustrates the topographic relationships of the various placental elements shown in fig. L. A maternal capillary (m.c.) containing four erythrocytes is shown in centre of figure. Its thickened endothelial wall contains numerous vacuoles and rests upon an irregular membrane of amorphous material (b.m.). A similar membrane surrounds the maternal giant cell visible at the top centre. To right and left of the giant cell lies the foetal syncytial trophoblast. Numerous mitochondria and dark-rimmed vacuoles appear within it. To extreme right of figure is a thin-walled foetal capillary (f.c.) separated from the syncytial trophoblast (s.t.) by cytotrophoblast (c.t.), the cytoplasm of which is less dense than that of the syncytium. A portion of the nucleus and cytoplasm of a cytotrophoblast is shown marginally in the lamella at top right of figure.

(magnification × 4,000)

FIG. U. Electron micrograph of a portion of the cat's lamella showing, at higher magnification, details of various placental elements. The maternal capillary (m.c.) containing a flocculent precipitate of plasma is shown at extreme left of figure. The thickened maternal endothelium (m.e.) has a lacy appearance caused by spaces or vacuoles filled with a flocculent precipitate similar to that in the capillary lumen. From the surface of the endothelium irregular projections extend into the lumen in fashions suggesting the enclosure of plasma into vacuoles by pinocytosis. To the right beneath the endothelium lies the thick, maternal basement membrane (b.m.). Note its discontinuity in places, and its irregular border where it adjoins the syncytium. At the base of the syncytium (bottom right), foot-like processes rest upon the foetal basement membrane (f.b.m.). These pseudopodia are surrounded by anastomosing extracellular space. A foetal capillary (f.c.) is illustrated at the upper right of the figure and adjacent to it is part of a cytotrophoblast (c.t.). Unlike the syncytium the cytotrophoblast lacks foot processes.

(magnification × 4,000)

FIG. V. Electron micrograph of rabbit's yolk-sac epithelium some hours after injection of Indian ink. Several dense clumps of carbon particles are present within the epithelial cells, as well as some loosely arranged masses above the nucleus. Within the nucleus at lower right of figure are several clumps of carbon, each of which is surrounded by a clear halo. Several possible nuclear pores are visible. At the upper right are several microvillous processes extending from the cell's surface. Note also the profusion of membrane-surrounded vacuoles which have a zone of increased density at their margins.

(magnification × 8,000)

FIG. W. Electron micrograph of rabbit's yolk-sac epithelium (endoderm) containing large inclusions after injection of cream. Surface of the endoderm cell, studded with microvilli, is at upper left of the figure. A thick amorphous basement membrane (b.m.) is evident at the base, separating the epithelial cytoplasm from the foetal connective tissue and its contained capillary (f.c.). Large vacuoles containing a reticulated osmiophilic amorphous material are distributed throughout the cytoplasm. Observe also that lipid-containing droplets of varying density are visible between the plasma membranes of adjacent cells.

(magnification × 5,000)

PLATE V, FIGS. X—CC

FIG. X. Electron micrograph of the labyrinth of the rat's chorioallantoic placenta at 17th day of gestation. Three cytotrophoblastic layers are seen intervening between a maternal blood space (m.b.s.) and the basement membrane (b.m.) and endothelium (e.n.) of a foetal capillary containing an erythrocyte. Nowhere is syncytium present.

(magnification × 9,000)

FIG. Y. Electron micrograph of the placental membrane of a rat at the 17th day of gestation, showing the cell bodies and nuclei of two cytotrophoblasts. In the cell cytoplasm, many ovoid mitochondria and skeins of endoplasmic reticulum are visible. Two foetal capillaries (f.c.) and a maternal blood space (m.b.s.) are also visible, separated by several sheets of trophoblast. The basement membranes of each of the foetal capillaries are clearly visible. The rat's placental membrane is thus not haemoendothelial but haemochorial, and is also cellular instead of syncytial.

(magnification × 4,000)

FIG. Z. Electron micrograph of a rat's placental labyrinth at full term (21st day of gestation), illustrating a foetal capillary (f.c.), a maternal blood space (m.b.s.), and the intervening tissue, consisting of endothelium, a basement membrane, and at least three sheets of cytotrophoblastic cytoplasm.

(magnification × 10,000)

FIG. AA. Electron micrograph through a human chorionic villus at term. The clear area at the upper left is the maternal intervillous space. The syncytium, the surface of which is provided with an abundance of microvillous processes, is denser at term than at earlier stages. The lighter cell, along the right border of the figure, which is interposed between the syncytium and the trophoblastic basement membrane, represents the remnants of the Langhans' cell (l.) of earlier stages. A dilated foetal capillary (f.c.) separated from the trophoblast by two basement membranes is visible in the lower portion of the figure.

(magnification × 4,000)

FIG. BB. Electron micrograph of the epithelial investment of a human chorionic villus of nine weeks, showing a profusion of microvilli projecting into the intervillous space (upper right). Some unusually large vacuoles (v.) are present in the apical syncytium. Portions of three cytotrophoblasts or Langhans' cells (l.) and their contained nuclei are visible in the lower portion of the figure. A syncytial nucleus distinguished by its greater density is also visible to the left of the cytotrophoblasts.

(magnification × 5,000)

FIG. CC. Electron micrograph through the edge of a human chorionic villus of nine weeks' gestation. The undulating surface of the syncytial trophoblast is beset with numerous microvillous processes which project into the intervillous space (m.b.s.) at the upper right. Besides a profusion of small spherical vesicles bounded by a distinct granular membrane, the syncytial cytoplasm contains some large apical vacuoles (v.) which Wislocki & Dempsey (1955b) interpret as a product of pinocytosis. Portions of three cytotrophoblasts or Langhans' cells (l.) are visible running diagonally across the lower left of the figure. These cells adjoin the foetal stroma and the foetal capillary (f.c.) at the lower left of the picture.

(magnification × 8,000)

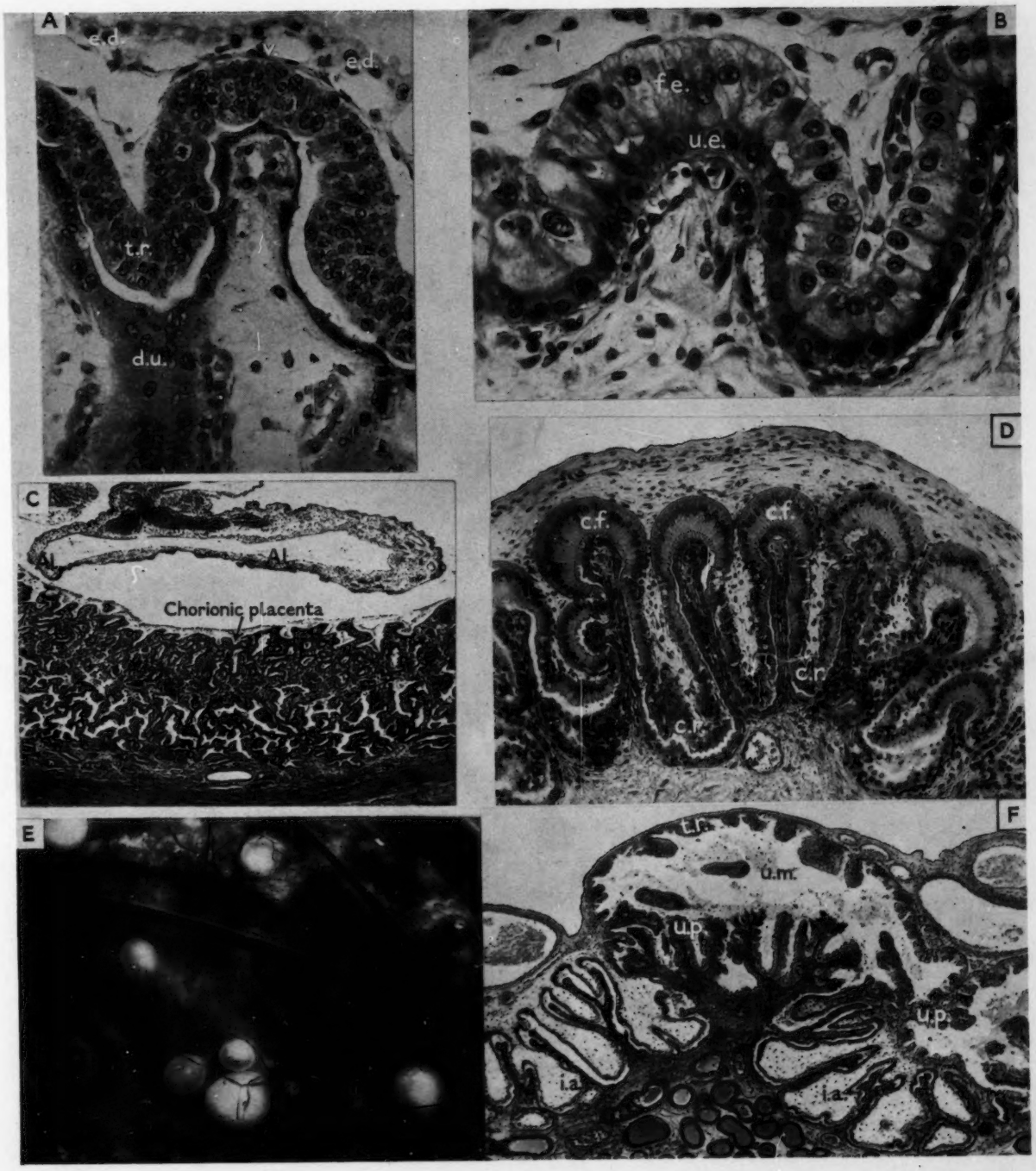
The electron micrographs have been supplied by E. W. Dempsey, Washington University School of Medicine, with the exception of Plate III: M, N and P, which were supplied by N. Björkman, Karolinska Institutet, Stockholm. The descriptions illustrating the fine structure of the placenta of the sow, the mare, the cat, the rat and man are based on the writings of Wislocki and Dempsey; that of the cow is based on the writings of Björkman and Bloom.

HISTOLOGY OF THE PLACENTA

(FIGS. A–CC)

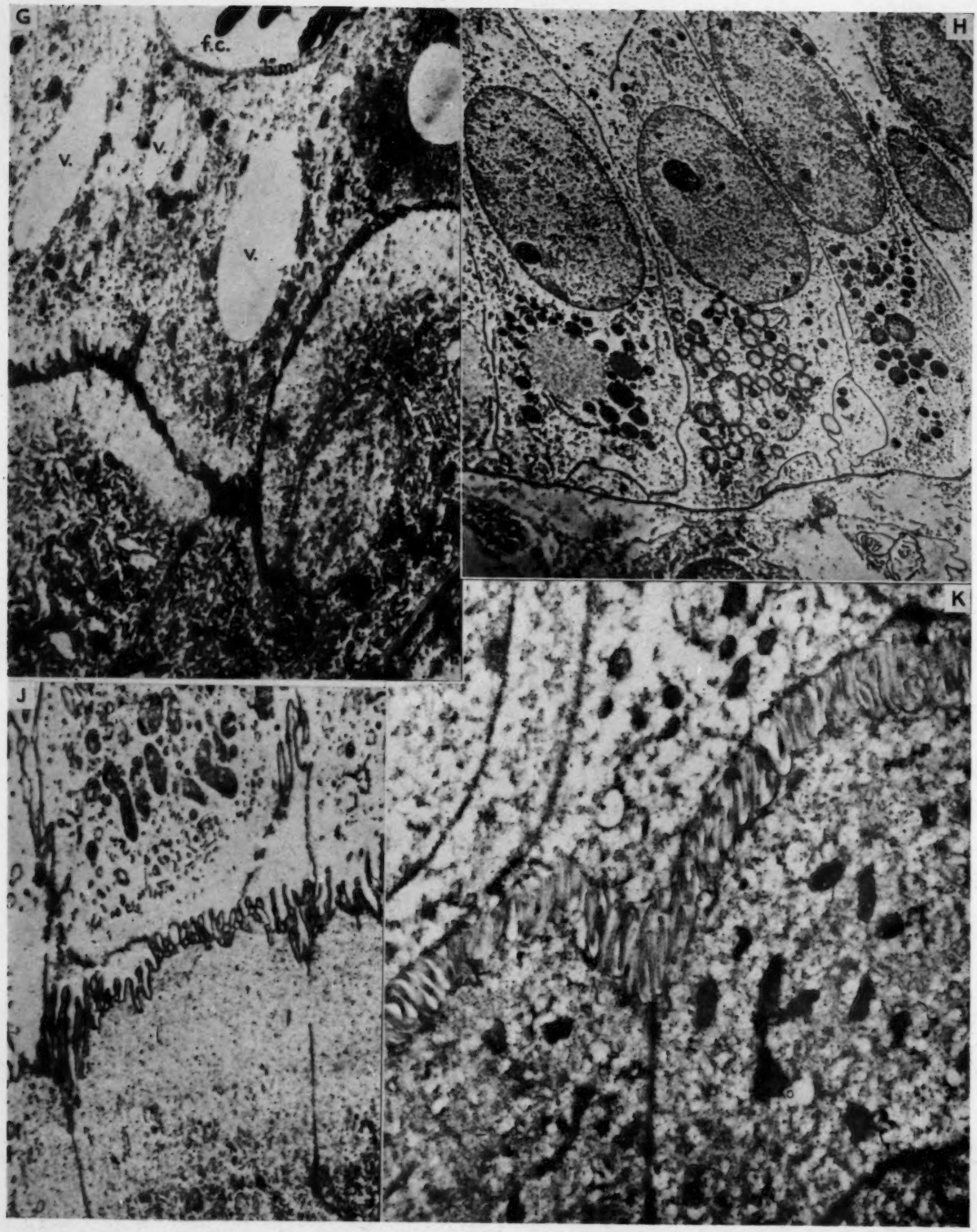
E. C. Amoroso

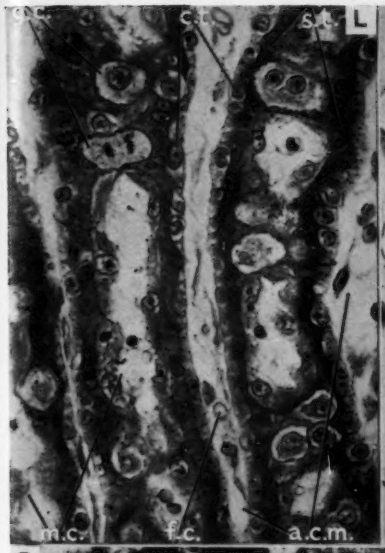
(page 81)



HISTOLOGY OF THE PLACENTA

(continued)

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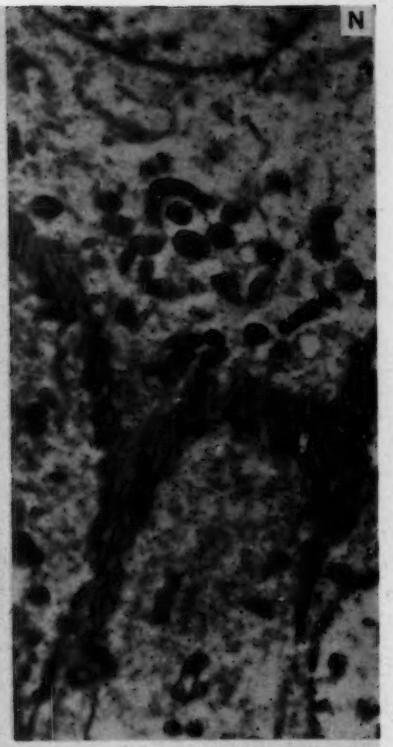
m.c.
f.c.
a.c.m.

M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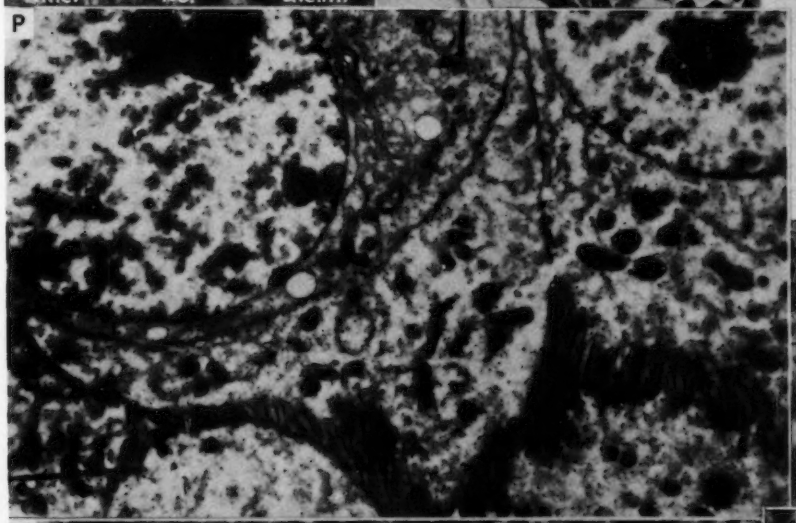
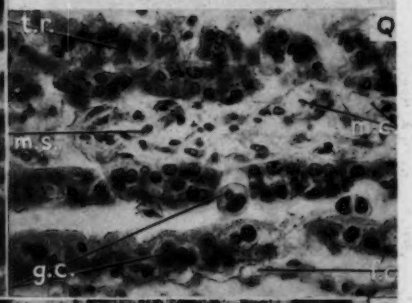
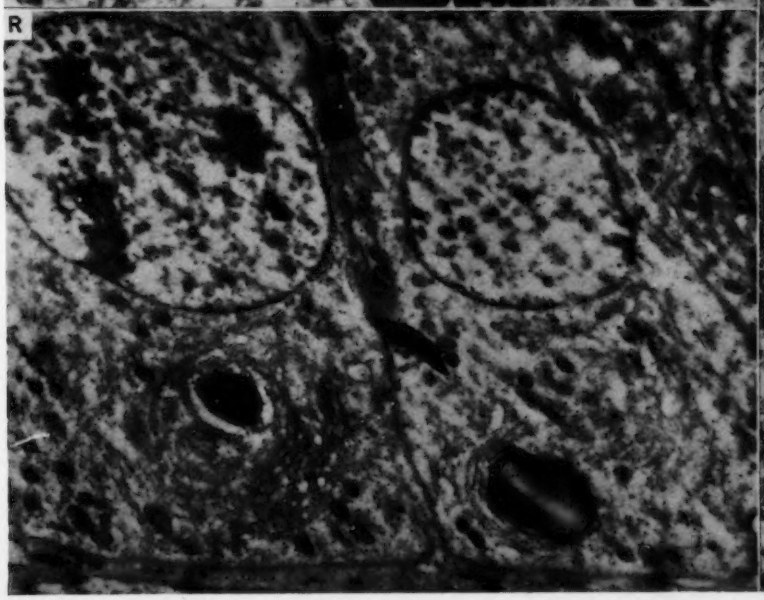
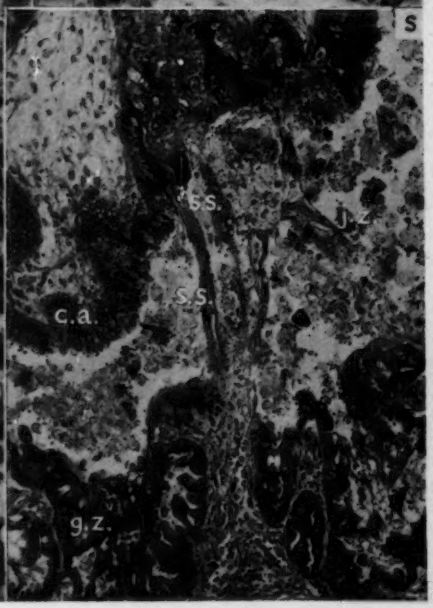

P

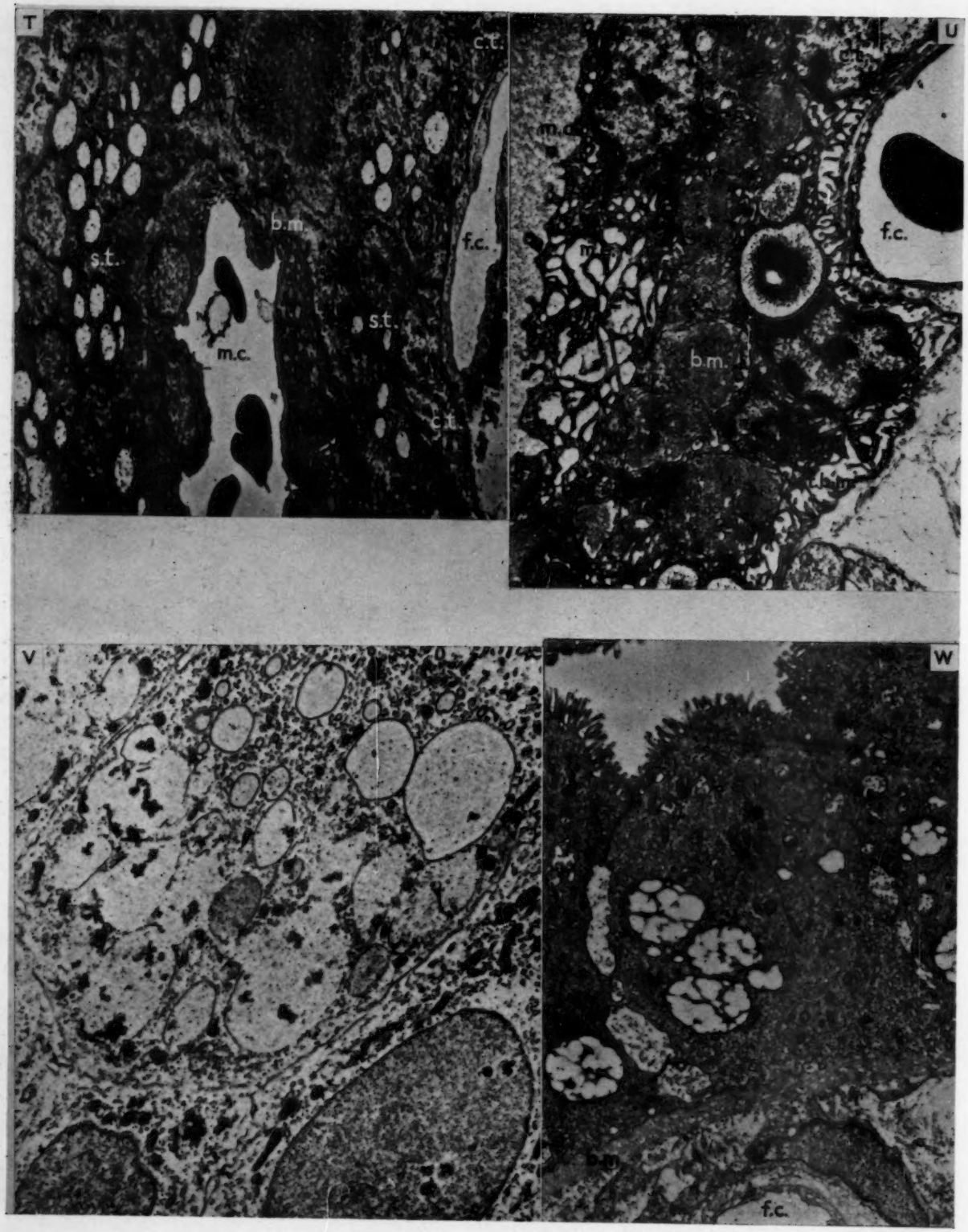

t.r.
Q
m.s.
m.c.
g.c.

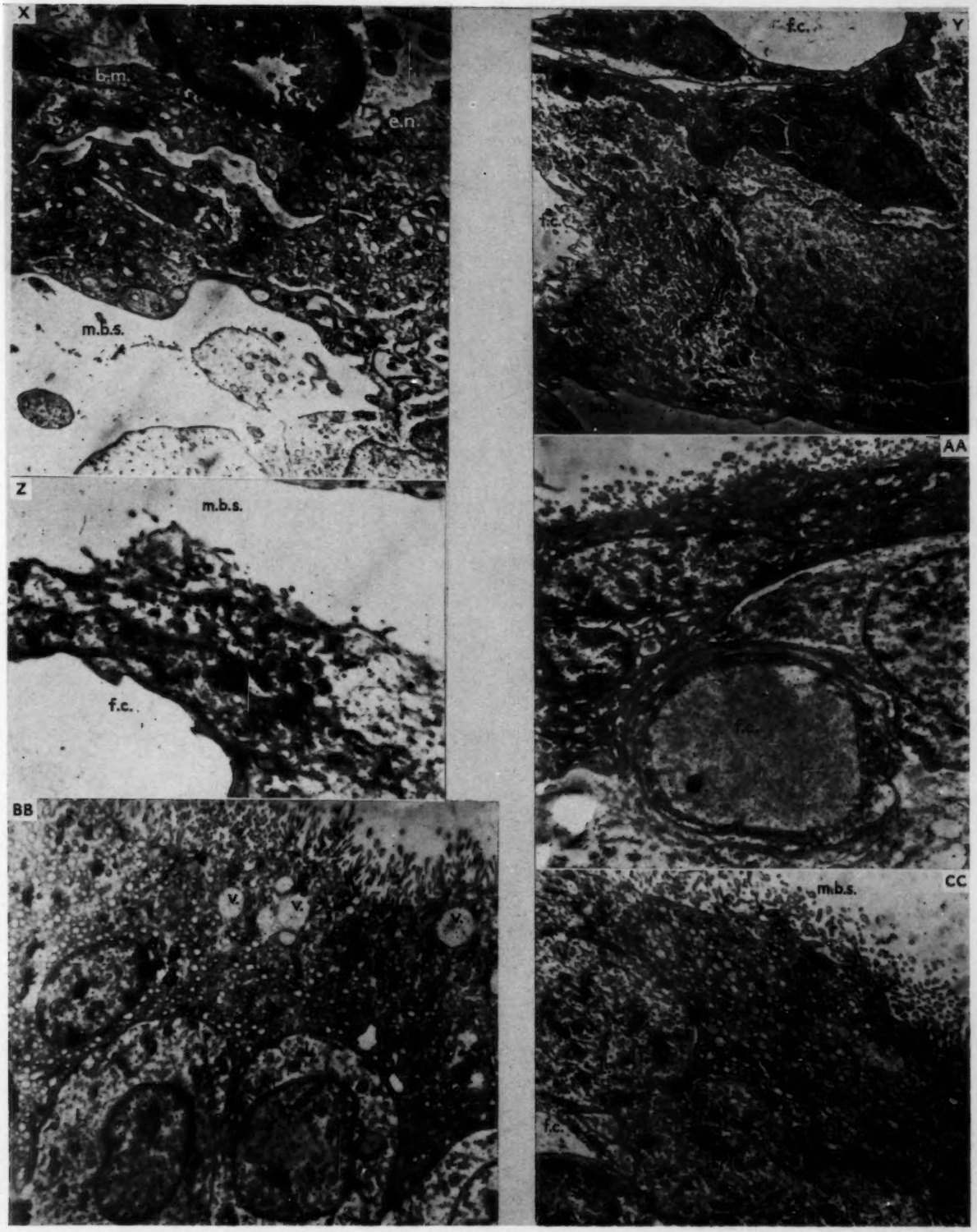

R


S
s.s.
l.z.
s.s.
c.a.
g.z.

HISTOLOGY OF THE PLACENTA

(continued)

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b.m.
e.n.
m.b.s.
Y
f.c.
f.c.
Z
m.b.s.
f.c.
AA
f.c.
BB
v.
v.
v.
CC
m.b.s.
f.c.

PLATE VI

FOETAL ENDOCRINOLOGY

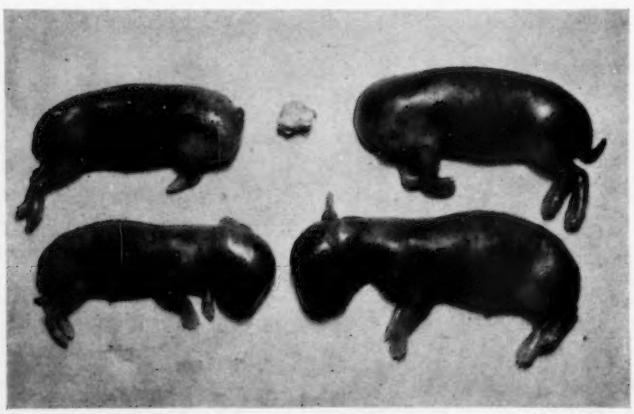
(FIGS. A, B)

R. Deanesly

(page 91)

A. Enlarged phallus and labia in a new-born girl. The mother was treated with 845 mg. testosterone and 112 mg. methyl testosterone during pregnancy.

Overzier (1957) by permission of Schweizerische medizinische Wochenschrift



B. A litter of 28-day rabbit foetuses of normal size, of which the upper two were decapitated in the uterus 9 days earlier, at the 2.5–3 cm. stage. In between lies the head of one, fixed in Bouin's fluid

Jost (1951b) by permission of Biologie Médicale

FOETAL ENDOCRINOLOGY

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1 Normal development and regression of foetal gonads and accessory organs
2 Effects of exogenous sex hormones on the developing foetus
3 Effects of foetal gonadectomy on the accessory organs
4 Effects of decapitation and destruction of the hypophysis on foetal growth, sex development and the adrenals
5 Development and regression of the human foetal adrenal
6 Experimental work on the foetal adrenal cortex
7 Foetal thyroid and foetal pituitary: effects of thyroxine and propylthiouracil
8 Conclusions
References

Experimental work over the last fifteen years has extended our knowledge of the development and interactions of the endocrine glands in the foetus but, taking into account the individuality of species and the changing phases of embryonic growth, it can be seen that research has only begun. The permeability of the placenta to sex steroid hormones is well established, but there are differences of opinion, and probably species differences, as regards placental transfer of the pituitary hormones. Some effects of maternal and exogenous hormones on the foetus, and of foetal hormones on development, will be described below; there is no satisfactory evidence that the foetal endocrines affect the mother.

Table I (from Jost, 1957a) lists experimental methods of studying foetal endocrinology. These involve problems of technique and interpretation, some of which are discussed by Moore (1950a) and by Wells, Cavenaugh & Maxwell (1954).

TABLE I. Main Techniques Used in Foetal Endocrinology (Adapted from Jost, 1957a)

Investigation of foetal endocrine gland secretions
Cytology or histochemistry of the glands themselves at various stages
Determination of the hormonal content of the gland at various stages
Observation of changes after hypophysectomy
Testing sensitivity to exogenous hormones
Metabolic study of the gland in situ
Establishing proof of a release of hormones in foetal blood
Investigation of foetal endocrine gland activity on target organs
Observation of pathological cases (e.g., freemartin, anencephalic monster)
Testing sensitivity of the target organs to exogenous substances
Determination of effect on target organs of removing or grafting the gland
Determination of effect of hypophysectomy on target organs

Malformation and failure to develop normally in experimental foetuses may be the result of operative interference with structures rather than deprivation of hormones. Wilson & Warkany (1948) showed that vitamin A deficiency in the rat could lead to malformations in the genito-urinary tract, but these may have been secondary to a disturbance of normal testicular function. Removal of a gland and its replacement by exogenous hormone will provide evidence of glandular secretion in a normal foetus, but in this type of experiment the stage of foetal development and the sensitivity of the male or female accessory organs, which may change rapidly, are extremely important (Jost, 1953b, 1958).

As will be seen (see section 7), the foetal pituitary influences the later differentiation of the other endocrine glands in the rat and rabbit, but little direct information is available for other mammals. Smith & Dortzbach (1929) assayed pig foetal pituitaries, and found gonadotrophic activity first at the 160–170-mm. crown-rump (C-R) stage. Nelson (1933) and others have examined such foetal pituitaries cytologically. Recent work with radioactive iodine on foetal thyroid activity in several species provides indications of the times at which pituitary thyrotrophic (thyroid-stimulating) hormone (TSH) is first produced. The development of pituitary activity in mammals is discussed in reviews by Moore (1950a), Jost (1951b, 1953b, 1956a) and Willier (1955).

1. Normal Development and Regression of Foetal Gonads and Accessory Organs

In many species, including man, the foetal gonads and accessory organs show a transitory stimulation usually attributed to the circulating placental oestrogens and gonadotrophins (Halban, 1904; Dobszay, 1938; Courrier, 1945; Parkes, 1954; Korpássy, 1959). This is followed by regression beginning either before or after birth. The pituitary in the pregnant woman (Bruner, 1951) and new-born child (Bahn, Lorenz, Bennett & Albert, 1953) appears to be gonadotrophically inactive.

The prostate shows a striking development in the last months of human foetal life; Korpássy (1959) states that after the postnatal degeneration the gland does not regain its birth-weight until ten years later. The epithelial metaplasia characteristic of the foetal prostate can be reproduced by oestradiol in the child up to one year old (Sharpey-Schafer & Zuckerman, 1941). Gillman (1948) and Albert, Underdahl, Greene & Lorenz (1953) found pre- and post-natal regression of the interstitial cells of the human foetal testis and attributed it to the disappearance of the chorionic gonadotrophin. Van Wagenen & Simpson (1954) noted foetal testicular development in the rhesus monkey 90–110 days pregnant, followed by postnatal regression. In the human female, too, there is a transitory development of the foetal gonads and of the uterus, vagina and mammary glands. Courrier (1945) recalls the findings of earlier writers, including Fraenkel & Papanicolaou (1938), Philipp (1938), and others. Milk secretion ("witches' milk") can often be expressed from the mammary glands of the new-born child, male or female.

Similar transitory development of the gonads and accessory organs in the cat and guinea-pig, in horses, cows, sheep and pigs as well as seals, elephants and giraffes, has been recorded by Courrier (1945), Parkes (1954), Amoroso (1955), Korpássy (1959) and Deanesly (1961). In the horse (Cole, Hart, Lyons & Catchpole, 1933; Parkes, 1954), male and female gonads

may be ten times as large in the 55–65-cm. C-R embryo as at parturition. They are 5–7 cm. long by about 4 cm. in diameter, weigh up to 150 g., and consist almost entirely of interstitial cells which regress in the last quarter of gestation.

2. Effects of Exogenous Sex Hormones on the Developing Foetus

It has been shown for many species (see Centre National de la Recherche Scientifique, 1950, and reviews by Burns, 1955; Jost, 1956b) that androgens may produce masculinizing effects, either given to the mother or directly to the developing foetus. The accessory male organs are stimulated but the ovaries are not affected. The results depend largely on the stage of development which the foetus has reached. The urogenital sinus in the normal male gives rise to urethra, prostate and seminal vesicles, and in the female it may form the urethro-vaginal canal, as in the rabbit, or two independent passages, the female urethra and the vagina. If the urogenital sinus is masculinized by androgens the accessory glands will develop, but not the vagina. Incomplete masculinization may also occur, the vagina being absent only at the posterior end. Burns (1956) observes that, in pouch opossums of the same litter, the genetic sex affects the extent of the accessory organ response to injected androgens or oestrogens, although qualitatively the effect is the same in the two sexes. With androgens the male sex primordia reach a greater size than those of the female, while with oestrogens the female sex primordia reach a greater size than those of the male.

The external genitalia are readily masculinized by androgens, e.g., in the foetal macaque when the mother received testosterone propionate (Van Wagenen & Hamilton, 1943). In recent times enlargement of the clitoris and labio-scrotal fusion has occurred in human infants when the mothers have received androgens (Zander & Müller, 1953; Overzier, 1957; and Plate VI: A) or androgenic progestagens (Moncrieff, 1958; Wilkins, Jones, Holman & Stempfel, 1958; Grumbach, Ducharme & Moloshok, 1959; Bongiovanni & McPadden, 1960; Grumbach & Ducharme, 1960; Jones & Wilkins, 1960). Other references may be found in these papers. From the available case reports it appears that 17-α-ethynyl-19-nortestosterone (Norlutin) has a greater androgenic action on the foetus than 17-α-ethynyltestosterone (Lutocyclol) and is comparable to methyl testosterone (Grumbach *et al.* 1959). Labio-scrotal fusion with a urogenital sinus occurred only when the androgen had been given before the 13th week of pregnancy (Wilkins *et al.* 1958). The external genitalia show considerable variation, but the masculinization is in general less than that which can be produced by virilizing adrenal hyperplasia. The androgenic progestagens appear to have a greater effect on the foetus than on the adult; there are indications that they are metabolized more slowly in the former (Grumbach *et al.* 1959). Courrier & Jost (1942) pointed out that 17-α-ethynyltestosterone could produce masculinization of the female rabbit foetus, and warned against its use in pregnant women.

Bongiovanni, Di George & Grumbach (1959) describe four cases of foetal masculinization of the external genitalia in which the mothers received no steroids other than oestrogens. The clitoris and labia were enlarged to a varying extent. Treatment had been started at different times, and the most conspicuous effects resulted from the administration of 10 mg. diethylstilboestrol daily between the fourth and ninth weeks. It seems possible that in these, and in the two other cases reported, the oestrogen stimulated a temporarily increased secretion of androgens by the foetal adrenals during a critical developmental period. Actually, in some of the previously reported cases, e.g., Hillman (1959), the mothers had received oestrogens in addition to progestagens. Many pregnant women have, however, received oestrogens in similar or larger amounts without any virilizing action on the offspring (Bongiovanni *et al.* 1959).

Oestrogens may lead to intersexuality in rat foetuses (Greene, Burrill & Ivy, 1939). The effects of oestrogens on foetal development are summarized by Jost (1958). In general they affect accessory organs, not gonads, but if given to the pouch opossum just after birth they can transform a testis to an ovo-testis (Burns, 1959).

3. Effects of Foetal Gonadectomy on the Accessory Organs

Bouin & Ancel (1903) suggested that the development of the accessory organs in the 3-cm. pig foetus was due to the secretions of the foetal testis, but it was primarily the papers of Keller & Tandler (1916) and Lillie (1917) on the freemartin which stimulated the study of sex development. Actually, the conditions found in the freemartin—atrophy of the ovaries of a female calf when joined by fusion of the membranes to a male twin, and regression of the Müllerian canals with development of the Wolffian canals and sometimes masculinization of the external genitalia—have not been reproduced in other species (Moore, 1950a; Jost, 1953b), and there is so far no confirmation of Lillie's original explanation that it was testicular hormone which caused the ovarian inhibition. Benirschke & Bloch (1960) extracted the testes of 100 cattle foetuses and found no recognizable C-19-steroids (ketosteroids). It is suggested that there may be a foetal testicular product able to influence development, different from that of the adult (Jost, 1957a). In three cases of twins, the females were established as freemartins at foetal lengths of 18, 18 and 25 cm. (corresponding to 100–120 days' pregnancy—gestation period, 280 days).

Moore (1941, 1943, 1950b) and Burns (1942) worked with the pouch young of the Marsupial *Didelphis*, gonadectomizing at day 20 and finding normal development of the accessory organs. Burns concluded that the gonads did not control sexual differentiation during the first 100 days of life. Jost (1947, 1953b, 1957a), however, obtained different results by gonadectomizing foetal rabbits at 19–24 days of pregnancy. He found that, if gonadectomy was done early enough, female accessory organs developed whatever the original sex, and he maintained that masculine development, including the disappearance of the Wolffian ducts, was effected through the testis secretion. Jost (1956b, 1957a) was unable, however, to reproduce the regression of the Müllerian ducts by testosterone propionate or several other androgens.

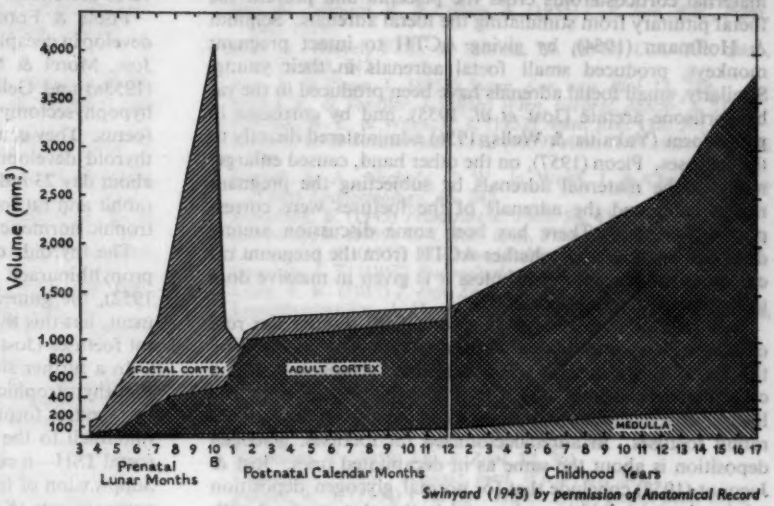
Gonadectomy on day 19 or day 20 suppressed the Wolffian ducts and prostate and permitted the development of female external genitalia; from the persistent Müllerian ducts a utero-vaginal tract developed. This result recalls the feminine conformation in human beings in which the gonads are congenitally absent. After unilateral castration, Jost (1953b) noted that the foetal testis had a local action on development which was absent on the castrated side. A crystal of testosterone propionate in the abdominal cavity of the castrated

foetus stimulated the Wolffian ducts, prostate and external genitalia.

Feminine differentiation of the tract in mice after *x*-ray destruction of the 13-day foetal testis was observed by Raynaud & Frilley (1947), and by Raynaud (1950). These experiments confirmed the theory of Wiesner (1934, 1935), based on gonadectomies of new-born rats, that the female type is the neutral or a-hormonal one.

It may be concluded that in small rodents, though not in the opossum, the foetal testis secretion controls the development of the male accessory organs, which can be sex-reversed only if the testis is removed at a sufficiently early stage. Removal of the ovaries leaves the development of the uterus and vagina unchanged.

FIG. 1. Prenatal and Postnatal Growth of the Human Adrenal, Plotted from Reconstructions of Serially-Sectioned Glands



Swinyard (1943) by permission of Anatomical Record

4. Effects of Decapitation and Destruction of the Hypophysis on Foetal Growth, Sex Development and the Adrenals

Raynaud & Frilley (1947) destroyed the hypophysis by irradiation in 13-day foetal mice and found that neither growth nor sexual differentiation was appreciably retarded. Jost (1948) decapitated 19-day rabbit foetuses and studied their further development. Some foetuses grew from a 2-g. headless body-weight to 17–25 g. by the 28th day; control foetuses seldom exceeded 30 g. (see Plate VI: B). The removal of the thyroid with the head in some foetuses did not affect growth. Foetal rats also grew almost normally after decapitation (Wells, 1947; Jost, 1948, 1956a; Jost & Picon, 1957).

Jost & Colonge (1949) decapitated rat foetuses at the onset of somatic sexual differentiation (day 16) and found the genital tract to be almost normal on day 21. Wells (1947, 1950) obtained similar results and also a reduction in the interstitial cells of the testis. Jost (1951a, 1951b) found abnormalities of the reproductive tract in the rabbit, however, when decapitation was done on day 19, and that these could be prevented by giving pregnant mare serum. He concludes that in the rabbit there is a hypophysial stimulation of the gonads about day 22–24, when the male structures are being established (Jost, 1953b).

5. Development and Regression of the Human Foetal Adrenal

A quantitative account of the well-known prenatal hypertrophy of the human adrenal described by Elliott & Armour (1911) and others is given by Swinyard (1943), who shows that the cortex grows very rapidly in the last three months of prenatal life and at birth constitutes 85% of the whole gland. In the first fortnight after birth it decreases in volume by 50%, and this involution, which is also found in accessory nodules, is not affected by prematurity, inanition or infection. By the end of the first year the foetal cortex residue is 15.9% of the whole gland (fig. 1).

The cytological details of the degenerative process, which may begin before birth, are described by Benner (1940), and the subject has been reviewed by Lanman (1953). Lanman (1957) found the adrenals normal up to the fourth month in anencephalic foetuses, but after about the 20th week they do not show the characteristic growth, and it must be assumed that a pituitary or hypothalamic factor is involved.

Benirschke, Bloch & Hertig (1956) discuss the maintenance and function of the foetal zone of the human adrenal with reference to the weak androgens found in it by Bloch, Benirschke & Rosemberg (1956), Dorfman (1957) and others. There is evidence that the foetal zone develops independently of adrenocorticotrophic hormones; it is possible that in later stages it is stimulated by luteinizing hormone produced by the pituitary under oestrogen stimulation. Early growth begins after the maximum period of chorionic gonadotrophin excretion. Bloch *et al.* (1956) found androstenedione and dehydroepiandrosterone, the concentration decreasing in the older foetuses. A sodium-retaining factor similar to aldosterone was also found in all age-groups, and traces of hydrocortisone were found at 16–21 weeks of gestation.

Lanman (1957) observes that the chimpanzee and potto among primates show a similar postnatal shrinkage of the adrenal, though the potto's adrenal is histologically different. Horse adrenals show no prenatal hypertrophy, but it has been described in the leopard, tiger, lion and elephant seal (see Deanesly, 1961).

6. Experimental Work on the Foetal Adrenal Cortex

Ingle & Fisher (1938), Walaas & Walaas (1944), and Davis & Plotz (1954) have all found that adrenalectomy of the pregnant rat leads to a hypertrophy of the foetal adrenals. Jost, Jacquot & Cohen (1955) noted that this enlargement did not occur in decapitated foetuses; this indicates that the foetal pituitary was involved. It had been previously shown (see Jost, 1953b) that the adrenals were conspicuously underdeveloped in decapitated foetuses but that they would respond to adrenocorticotrophic hormone (ACTH) (Kitchell & Wells, 1952). Yakaitis & Wells (1956) found that hypophysectomy of the pregnant rat after day 12 did not affect the foetal

adrenals unless the foetuses were also decapitated. From these experiments and others it has been deduced that, normally, maternal corticosteroids cross the placenta and prevent the foetal pituitary from stimulating the foetal adrenals. Schmidt & Hoffmann (1954), by giving ACTH to intact pregnant monkeys, produced small foetal adrenals in their young. Similarly, small foetal adrenals have been produced in the rat by cortisone acetate (Jost *et al.* 1955), and by cortisone in pellet form (Yakaitis & Wells, 1956) administered directly to the foetuses. Picon (1957), on the other hand, caused enlargement of the maternal adrenals by subjecting the pregnant rat to cold, and the adrenals of the foetuses were correspondingly small. There has been some discussion among different workers as to whether ACTH from the pregnant rat can cross the placenta, but unless it is given in massive dose this probably does not occur.

Jost (1957a) and Jost & Jacquot (1958) discuss the role of the pituitary and adrenals in the synthesis of glycogen by the foetal liver. In the foetal rat, liver glycogen shows a clear increase around day 19; in decapitated foetuses the liver glycogen is reduced, but not by as much as in decapitated rabbit foetuses. In adrenalectomized rat foetuses, glycogen deposition is about the same as in decapitated ones. Jost & Jacquot (1958) conclude that for normal glycogen deposition a hypophysial (rabbit) or placental (rat) factor must act with the adrenal corticoids.

7. Foetal Thyroid and Foetal Pituitary: Effects of Thyroxine and Propylthiouracil

Morphological and biochemical data indicate that the thyroid becomes active before birth. Uptake of ^{131}I occurs on day 18–19 in the rat (Gorbman & Evans, 1943), about day 52 in the sheep (Barnes, Warner, Marks & Bustad, 1957), about day 50 in the pig (Rankin, 1941) and rather later in the cow (Koneff, Nichols, Wolff & Chaikoff, 1949). Development of the thyroid in the rabbit has been studied by Waterman & Gorbman (1956) and by Jost (1953a, 1957b, 1958) and Geloso (1958). The thyroid becomes functional in or just before the last week of gestation. In man (Chapman, Corner, Robinson & Evans, 1948; Speert, Quimby & Werner, 1951; Hodges, Evans, Bradbury & Keettel, 1955; Logothetopoulos & Scott, 1956) it appears between the 12th and 14th week. Korpássy (1943) and Eickhoff (1958) examined the pre- and post-natal human thyroid and concluded that it was very active near the time of birth.

Carr, Beierwaltes, Raman, Dodson, Tanton, Betts & Stambaugh (1959) studied the effect of the maternal thyroid on foetal thyroid function in man and dogs, and concluded that hypothyroidism in the mother was not associated with the same condition in the foetus. Similarly, Peterson & Young (1952) found normal foetal thyroids in the young of guinea-pigs, thyroidectomized and treated with thyrotrophic hormone. Heggestad & Wells (1954) and Sobel, Hamburgh & Koblin (1960) found that in the rat the thyroid develops normally in the absence of the maternal pituitary (from day 12 of gestation).

Foote & Foote (1949) observed failure of the thyroid to develop in decapitated (hypophysectomized) hamster embryos. Jost, Morel & Marois (1949), Sethre & Wells (1951), Jost (1953a) and Geloso (1956, 1958) have studied the effects of hypophysectomy by decapitation on the rabbit and rat foetus. They concluded that in rabbits decapitated on day 19, thyroid development at first proceeded normally, but from about day 23 iodine uptake was inhibited. In the decapitated rabbit and rat foetus the thyroid can be stimulated by thyrotrophic hormone.

The thyroids of guinea-pig and rat foetuses enlarge when propylthiouracil is given to the mother (Peterson & Young, 1952), the guinea-pig foetal pituitary also showing enlargement, but this thyroid reaction does not occur in decapitated rat foetuses (Jost, 1957b).

In a further study, Knobil & Josimovich (1959) conclude that thyrotrophic hormone does not cross the placenta in the rat; goitre formation in the foetus after feeding propylthiouracil to the mother is due to an increased secretion of foetal TSH—it occurs in the absence of the maternal pituitary. Suppression of foetal TSH is obtained by giving thyroxine to pregnant rats (Knobil & Josimovich, 1959; Noumura, 1959; Sobel *et al.* 1960). Thyroxine also retards thyroid development if given directly to the rat foetus, and the effect can be prevented by injecting thyrotrophin at the same time (Hwang & Wells, 1959). It may be concluded that, although the developing thyroid is not necessary for growth in rat or rabbit (Jost, 1957a), it does become active in the foetus under the stimulation of injected thyrotrophin or of the foetal pituitary, which itself can be affected by exogenous thyroxine, propylthiouracil, and other substances, passing across the placenta.

8. Conclusions

In many animals, especially those with a long pregnancy, the gonads and accessory organs show a remarkable prenatal growth and differentiation, followed by rapid regression after birth or sometimes earlier. This temporary enlargement can be attributed to the circulating oestrogens and gonadotrophins of placental origin, and it is doubtful if it has any functional significance.

The experimental operations by Jost, Wells and their collaborators, on pregnant rabbits and rats, and on foetuses in utero, have shown that in these animals the pituitary and testes become active before birth and play a decisive part in differentiation. Thyroid and adrenal development is controlled by the foetal pituitary. The developing foetus at certain stages can react to exogenous hormones, which reach it directly or via the placenta. The fact that the endocrine glands are active in the foetus carries no implication that their secretions can affect the maternal physiology.

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PROTEIN TRANSFER ACROSS THE FOETAL MEMBRANES

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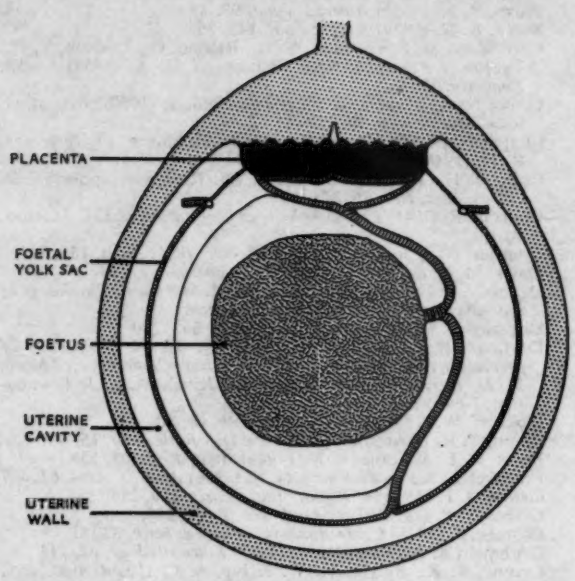
Foreword
1 Immunological status at birth
2 Characterization of the serum components
3 Development of the serum components
4 Selection
References

Foreword

The rabbit and the guinea-pig resemble man and the monkey in that their young are born with their full equipment of passive immunity, and this is not augmented after birth to any significant extent. They are the only animals, other than monkeys, at present known to resemble man in this respect. Although there is some transmission of passive immunity before birth in other animals, most of this transmission (in rats, mice and dogs) or all of it (in ruminants, horses and pigs) occurs by way of the milk and the gut of the young animal after birth. Hence the rabbit is the experimental animal of choice for studying the route of transmission of antibodies from the maternal circulation across the foetal membranes to the foetal circulation. It has been shown that this is by way of the uterine cavity and the vascular foetal yolk-sac splanchnopleure (see fig. 1) and not across the chorio-allantoic placenta. This is comparable to the route in birds, in which the maternal antibodies are secreted into the yolk of the egg and subsequently transmitted to the circulation of the chick by the yolk-sac wall (Brierley & Hemmings, 1956). It resembles transmission in other mammals after birth by way of the milk, in so far as the absorptive surface is an endodermal membrane, morphologically part of the gut. The route cannot be similar in man, however, because the yolk sac of the foetus is a rudimentary structure and the chorion completely surrounds the conceptus and must be traversed. However, all that was known as regards the route of transmission of passive immunity has been reviewed recently and need not be detailed here (Brambell, 1958). What is relevant is that the absorptive membrane, the yolk sac of the foetal rabbit, is readily accessible to experimental exposure to immune or isotopically labelled serum protein preparations, simply by injecting these into the cavity of the gravid uterus.

Studies using this technique have shown that transmission is a highly selective process. All antibodies are not transmitted equally readily. Antibodies formed in different species to a given antigen may be transmitted at very different rates,

FIG. 1. Diagrammatic Cross-Section of the Gravid Uterus of a Rabbit, to Show the Arrangement of the Foetal Membranes



The endoderm of the yolk sac is cross-hatched. The chorion, in solid black, joins the margin of the yolk sac to the placenta. The thin amnion surrounds the foetus and is separated from the yolk sac by the exocoel. From the umbilicus the allantoic stalk runs both to the allantoic vesicle attached to the inner surface of the placenta and also to the yolk sac.

as may antibodies to different antigens formed in a given species. Even two antibodies in the serum of the same individual may be transmitted differentially. Moreover, the several fractions of the serum proteins are each transmitted at a characteristic rate and this is not simply related to molecular size: for example, γ-globulin is transmitted more readily than albumin. Further, the large polypeptide fragments of a pepsin- or papain-treated antibody are transmitted less readily than the intact molecules (Brambell, Hemmings & Oakley, 1959; Brambell, Hemmings, Oakley & Porter, 1960). The selective transmission of proteins by the yolk sac of the foetal rabbit provides a very promising approach to study of the significance of the molecular configuration of proteins in cell function. It has led to the finding that other endodermal membranes, notably the gut of the young rat (Halliday & Kekwick, 1960) and the yolk sac of the chick, are selective also, so that several experimentally accessible membranes are available for the study of the process. Such work involves the utmost refinement of serological techniques, and progress waits in large measure upon advance in protein chemistry, fortunately rapid at the present time.

The extensive literature of the transmission of passive immunity in man has been confused by the wide differences observed in the efficiency with which different antibodies are transmitted from mother to infant. There are numerous instances, and that between complete and incomplete Rh antibody jumps to mind. The significance of these differences

has not been understood hitherto, but it is clear now that they are other examples of selective transmission of proteins. The present paper reviews our knowledge of selective transmission of serum proteins in the rabbit and in man. It will be evident to the reader that, although the problem is far from solved, the outlines of a pattern are beginning to emerge.

1. Immunological Status at Birth

Since the pioneer observation of Ehrlich (1892) that young mice are protected against the toxic action of rice protein by suckling an immunized mother, the curious state of dependence of mammals and other vertebrates upon maternal immunity has been widely studied. The reticulo-endothelial system is late in maturing, the plasma cell being the last element to reach full function, usually well after birth. Maternal antibody certainly preserves the young against pathogenic affront while this development takes place, but it may be unwise to emphasize this aspect of the relationship. It may be equally rewarding to regard the immune response as delayed in its development in order that the young may benefit for a longer period by the supply of ready-made macromolecules. As in many other aspects of mammalian development, the causal relations are likely, when established, to be less obvious than may appear at first sight.

The confrontation of two genotypes in mammalian pregnancy is a fascinating situation. The hazards of being pregnant perhaps have been traditionally over-emphasized. The hazards of being gestated, however, are very real though not so readily apparent, and largely remain to be studied. A part of the risk is associated with the transfer of maternal plasma protein, which must normally contain substances active as enzymes, iso-antibodies to blood-group substances or to γ-globulin, cytotoxins and possibly protein hormones likely to be of no great benefit to the foetus. Cord blood plasma is far superior to adult plasma as a medium for cell culture in vitro. The foetus according to its species receives a greater or less contribution to its plasma, but in each case of which we have knowledge this contribution is a selective one. Much of the maternal protein available is in fact excluded.

For obvious reasons the study of this phenomenon was restricted almost exclusively to the transfer of antibodies, until recent years. Reviews of this work are given by Vahlquist (1952), Edsall (1956) and others cited by Brambell & Hemmings (1960). The location of transfer processes, the histological route, and the period of their occurrence vary in different species. Thus (see Table I) the ungulates are born with no antibody and little γ-globulin, but derive these in one massive dose from the colostrum, the small intestine being permeable to protein for a brief period after birth, but apparently not selective. In the rabbit and guinea-pig all transfer occurs before birth and is selective. The maternal supply is secreted into the lumen of the uterus and is absorbed by a well-vascularized membrane, morphogenetically part of the gut wall, called the yolk-sac splanchnopleure. This membrane, which forms the outer cover of the conceptus, is covered by a low columnar epithelium of endoderm cells (fig. 1). Under the electron microscope these cells are seen to possess the micro-villous cell border and associated cytoplasmic vacuolation which are indicative of intense droplet ingestion (Dempsey, 1953). In some cases, labelled material has been demonstrated within these vacuoles after experimental administration into the cavity of the uterus.

TABLE I. Time of Transmission of Passive Immunity in Mammals

Species	Transmission of passive immunity	
	Prenatal	Postnatal
Ox, goat, sheep	0	+++ (36 hr.)
Pig	0	+++ (36 hr.)
Horse	0	+++ (36 hr.)
Dog	+	++ (10 days)
Mouse	+	++ (16 days)
Rat	+	++ (20 days)
Rabbit	+++	0
Guinea-pig	+++	0
Man	+++	0

Brambell (1958) by permission of Biological Reviews

The mouse and rat also transfer protein by this route, but the contribution made to the foetal plasma is small in comparison with the supply gained from colostrum and milk after birth. Absorption from the gut is selective in mouse and rat.

In man there is some evidence that the transfer is effected by way of the chorio-allantoic placenta. The yolk-sac route is hardly likely to be implicated, for this organ is but a vestige in the latter part of gestation. Comparative evidence suggests absorption by way of the amnion and gut as a possible alternative (Brambell, Brierley, Halliday & Hemmings, 1954); yet oesophageal atresia does not impede the transfer (Wasz-Höckert, Wager, Hautala & Widholm, 1956). The foetal liver and placenta incorporate labelled glycine into albumin, α- and β-globulins (Dancis, Braverman & Lind, 1957), and the former organ, as early as the third month, may be able to meet the foetal requirement of all protein save the γ-globulin. In the rhesus monkey it has been shown that labelled maternal albumin and γ-globulin reach the foetal circulation and amniotic liquor from the maternal circulation (Bangham, Hobbs & Terry, 1958; Bangham, 1960). In two cases where maternal protein was injected into the amnion there was little evidence of its reaching the foetal blood although it did reach the foetal gut.

2. Characterization of the Serum Components

The functional complexity of the serum proteins has long been recognized, but our knowledge of it depends on the techniques of separation and characterization. The classical techniques of ultracentrifugal analysis and free boundary electrophoresis have shown that at least twenty components are normally present in human serum (Wallenius, Trautman, Kunkel & Franklin, 1957).

Separation by the methods of salting out, and of cold precipitation with ethanol (Cohn, Strong, Hughes, Mulford, Ashworth, Melin & Taylor, 1946) or ether (Kekwick & Mackay, 1955), produce fractions which are found to be complex when analysed in the ultracentrifuge or electrophoretically (Oncley, Scatchard & Brown, 1947). It is clear that, in comparison with the structural diversity possible to molecules of the size of these proteins, such methods are still relatively crude, and not likely to lead to separations of molecular kinds which will parallel their biological properties. Thus an apparently single function, for example Rh incomplete agglutination, may be found in a range of molecular

types (Abelson & Rawson, 1959), while some functions of presumptively high survival value to the organism are associated with definable fractions present at low absolute concentration—for example, properdin (Lepow, Pillemer, Schoenberg, Todd & Wedgwood, 1959) and caeruloplasmin (Uriel, Götz & Grabar, 1957; Deutsch, 1960).

In recent years successive technical improvements have given easier access to such minor components. Zone electrophoresis in its various forms has provided powerful preparative techniques (e.g., see Porath, 1956) and microanalytical methods of considerable precision: it combines flexibly with chemical and serological methods. Thus the starch-gel procedure used to determine the haptoglobins by Smithies (1955) gives high resolution, particularly of the α- and β-globulins, and has been employed extensively. A weapon of great sensitivity is available from immunology, and one of long standing, in that animals may readily be persuaded by inoculation with foreign protein to produce specific reagents which serve to demonstrate that protein. Thus serological identification of components as antigens precipitating with immune sera in gel diffusion has provided a powerful method (Oudin, 1952), particularly in the form of immuno-electrophoresis (Grabar & Williams, 1953), by which 15 components of normal immune serum were distinguished in the first place (Williams & Grabar, 1955)—a number subsequently increased to 18 (Gugler, von Muralt & Bütler, 1959) and recently to more. The chromatographic procedures have been greatly strengthened by the development of the cellulose exchangers (Peterson & Sober, 1956) which have high loading capacity and good resolving power. They have proved particularly useful in the fractionation of the γ-globulin antibodies (see, e.g., Humphrey & Porter, 1957; Abelson & Rawson, 1959; Fahey & Horbett, 1959). Since the protein is not subjected during fractionation to drastic changes of ionic concentration, pH or temperature, the integrity of the product is far better preserved than by the older methods, and probably compares well with zone electrophoresis.

The newer techniques are typified by the simultaneous use of two or more criteria and are thus a step toward matching the complexity of the protein molecule. Thus, after electrophoresis in gel, components identified by serological criteria show in almost all cases the same mobility as in the free boundary method. Again, the serological identification of components of like mobility parallels the separation by molecular weight in the ultracentrifuge. Thus the β_2M band of immuno-electrophoresis is produced by the macro-globulin of Waldenström's syndrome (Waldenström, 1952) and so is equated with part of the rapidly sedimenting component of normal serum (Burtin, Hartmann, Heremans, Scheidegger, Westendorp-Boerma, Wieme, Wunderly, Fauvert & Grabar, 1957). This component is now known to contain, for example, the cold auto-antibodies of acquired pernicious anaemia (Mehrotra, 1960). Similarly, differences of antigenicity have been exploited in distinguishing components of molecular weight of approximately 160,000 from those of approximately 900,000 in human γ-globulin (Franklin & Kunkel, 1957; Chan & Deutsch, 1960)—this has been of considerable value in determining that the larger molecule is not simply a polymeric form of the smaller. Molecular size will hereafter be referred to in terms of the Svedburg unit of sedimentation, the normally predominant γ-globulin of 160,000 molecular weight having a sedimentation constant of approximately 7S. γ-Globulin—regarded as antibody—can obviously be subdivided into a large number of classes, limited possibly by the organism's capacity to respond, possibly only by the experimenter's ability to devise specific simple antigens with which to challenge that capacity. Even such an apparently homogeneous antigen as human serum albumin has been shown to give rise to multiple antibodies (Lapresle & Durieux, 1957). At present the hypotheses of antibody production, although numerous and indeed fertile, do not readily suggest critical tests. They are reviewed by Burnet (1959) and Bussard (1959). However determined, it is clear that antibody may exhibit a wide range of molecular structure which will vary with the immunological experience of each individual. At the same time the antigenic specificity of the molecule is invariable, and relatively simple. Thus, the normal immuno-electrophoretic γ-globulin band extends through γ_2-, γ_1-, β_2- and β_1-zones and forward into the α_2-zone (Williams & Grabar, 1955), apparently as a single antigenic specificity, indicating a continuous variation in mobility. The need to reconcile variability of structure as antibody with this constant specificity as antigen led Pauling (1940), among others, to suggest a "two-part" molecule for γ-globulin. The key to this situation is perhaps given by the finding that rabbit γ-globulin breaks up, by enzyme action and rupture of S–S bonds, to three constituent parts, two of which are "univalent" type antibody, each with a single combining site, while the third alone retains the original antigenic specificity of the whole molecule but lacks antibody activity (Porter, 1958, 1959; Nisonoff, Wissler, Lipman & Woernley, 1960). Similar fragmentation is brought about by either papain or pepsin so that the point of cleavage is to this degree independent of the enzyme specificity. Of the three types of fragment, one only, that bearing the antigenic specificity, passes from mother to foetus at the rate characteristic of the whole molecule; the antibody fragments, despite their low molecular weight, are relatively excluded (Brambell *et al.* 1959). Rabbit γ-globulin, although diverse in molecular size (Kabat, 1939; Palc, 1939; Stelos, 1956), is apparently constant in the sequence of the last five amino acids of the amino end of the peptide chain (Porter, 1950; McFadden & Smith, 1955). Of the human γ-globulin, it appears that the 7S Rh antibody which agglutinates erythrocytes in albumin solution can be dissociated into univalent Coombs' sensitizing antibody by papain digestion (Chan & Deutsch, 1960).

The macro-globulin antibodies can also be decomposed to smaller fragments, simply by rupture of the S–S bond with mercaptan (Fudenberg & Kunkel, 1957), the fragments being of approximately 7S (Deutsch & Morton, 1958). They may retain antibody activity in modified form; thus saline agglutinating Rh antibody, after mercaptan dissociation, acts as a Coombs' test sensitizing antibody (Chan & Deutsch, 1960). These fragments cross-react with antibody specific to either the normal 7S or 19S molecule, but in each case lack the full antigenic specificity (Franklin, 1960). They cannot thus be regarded as a normal adult serum component, nor can the macro-globulin be considered, according to the above-named workers, to be a polymer of the normal 7S molecule. It is not yet apparent, however, whether such dissociation of the macro-globulin does, or does not, occur in vivo.

3. Development of the Serum Components

Though a considerable amount of work has been done on the postnatal serum in several species of mammals, develop-

mental stages have received little attention. The literature is admirably reviewed by Kekwick (1959). The serum of the human foetus, somewhat unusually, is better known than any other.

The four major components have been shown to be present in man at three months' gestation; their relative concentrations change steadily up to full term. The absolute concentrations of α- and β-globulin remain steady, while albumin and γ-globulin follow approximately parallel curves (Moore, Du Pan & Buxton, 1949). By full term, the foetal γ-globulin concentration considerably exceeds the maternal. Other studies of cord and maternal blood at birth confirm this finding for American and European samples, but a study of African babies shows a γ-globulin level below the maternal.

The antibody content is known to rise during the latter part of gestation. Antistaphylolysin and antistreptolysin titres are found between 16 and 22 weeks' gestation, but are then low and reach equality with the maternal level toward full term (Vahlquist, Lagercrantz & Nordbring, 1950). Diphtheria antitoxin concentrations in foetal blood, relative to maternal, increase regularly with body-weight in premature deliveries (Osborn, Dancis & Rosenberg, 1952) and vary from equality to 2.2 times that of the mother at full term (Barr, Glenny & Randall, 1949). This finding is similar to those of many other workers and appears to hold also for tetanus antitoxin, antistaphylolysin, antistreptolysin O and *Haemophilus pertussis* agglutinin. In a number of anti-viral antibodies a similar ratio of foetal and maternal concentrations has been observed. (For references see Brambell & Hemmings, 1960.)

At full term the α-globulin approximates to the normal adult concentration, while the β-globulin falls somewhat below this (Longsworth, Curtis & Pembroke, 1945). The maternal levels of these proteins rise throughout pregnancy, in contrast with the foetal levels. Correspondingly, the maternal serum is considerably richer in lipoprotein and α- and β-glycoprotein (Kent & Gey, 1960). The development of the foetal antigenic components during gestation has been followed by immuno-electrophoretic analysis (Scheidegger, Martin & Riotton, 1956). At birth two components of the β-globulin are still absent: β_2A and β_2M (Hitzig, 1957; Scheidegger & Du Pan, 1957). The latter is a macro-globulin, and may be antibody. It is possible that its early development may be stimulated by antigenic challenge of premature infants, which is reported to lead to the production of a macro-globulin antibody of γ_1 mobility (Smith, 1960).

After birth a steep rise of α- and β-globulin concentration occurs in the first few weeks (Moore *et al.* 1949). Albumin also continues to rise slowly, but there is a logarithmic decline of γ-globulin throughout the first few months of babyhood, continuing according to Knapp & Routh (1949) to a minimum at about one year. The antibody concentrations again parallel this fall. Diphtheria antitoxin of maternal derivation declines with a half-life of 4.5 weeks (Barr *et al.* 1949), and Rh incomplete antibody with a half-life of 30 days (Wiener, 1951), while poliomyelitis antibody has been variously reported at 21 days (Perkins & Yetts, 1958), 25 days (Gelfand, Fox, LeBlanc & Elveback, 1960) and higher values. The rate of elimination of these molecules keeps pace with the decline of the total γ-globulin concentration, but it is considerably lower than that normal in the adult. A similar extension of the half-life of passively acquired globulin has been observed in the young rat (Hemmings, unpublished work). This is possibly typical of the phase of slow development of the immune response, though it is not clear why the inability to synthesize antibody should be associated with a compensating reduction of catabolism. Production of antibody during this period is poor in man (Osborn, Dancis & Julia, 1952), in rabbits (Freund, 1930) and in piglets (Šterzl, Kostka, Mandel, Říha & Holub, 1960), and there is a correlated lack of autogenous globulin. The lymphoid tissues are only slightly developed and especially are lacking in plasma cells. In cases of congenital hypo-γ-globulinaemia, where the capacity to form antibody is never developed, there is an associated lack of plasma cells; passively acquired antibody enjoys a prolonged half-life, as it does in the normal suckling (Bridges, Condie, Zak & Good, 1959).

Comparable studies of the foetal stages in other species, although badly needed, are very few. Moore, Shen & Alexander (1945) give data for the pig, Brambell, Hemmings, Henderson & Kekwick (1953) and Myant & Osorio (1959) for a restricted stage in the rabbit.[1] We ourselves are not aware of any prenatal data on the guinea-pig, rat or mouse.

4. Selection

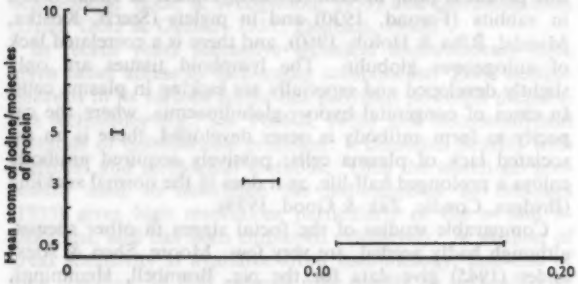
It has long been accepted that not all antibodies present in maternal serum reach the foetus in equal amount. Thus, for example, the typhoid H agglutinin has been reported to reach equal levels, whereas the O agglutinin is excluded (Timmerman, 1931). The blood-group antibodies have provided particularly elegant demonstrations of the differential transmission of two antibodies in the one maternal serum. Thus, two cases of haemolytic disease due to A and B sensitization, with O mothers versus A infants, permitted Wiener & Sonn (1946) to compare the foetal and maternal titres of complete and incomplete β-iso-agglutinins. The incomplete β-iso-agglutinins reached equality: the complete ones reached $\frac{1}{8}$ and $\frac{1}{64}$ of this level.

Inter-specific selectivity, although hardly a normal foetal experience, emphasizes the fundamental character of the mechanisms involved. Thus Hartley (1948) drew attention to the case of twins succumbing to diphtheria shortly after birth, indicating that refined horse antitoxin administered in large amounts to the mother a few days before birth had not reached them in significant amounts (Chesny, 1945).

During investigation of the process of prenatal transfer in the rabbit, a similar ability to distinguish heterologous molecules was discovered (Brambell, Hemmings, Henderson & Rowlands, 1950). When rabbit and bovine antibodies are presented together to the foetal absorptive surface, the homologous appear in the foetal circulation, the heterologous do not. Studies with labelled globulin preparations showed that, in fact, protein of both species is taken up by the endoderm cells, and that the exclusion from the circulation of the heterologous protein is relative, not absolute; small quantities of antigenically bovine protein, which also retains its antibody specificity, do in fact regularly reach the foetal circulation (Hemmings, 1956, 1958). The response to foreign proteins is a graded one, according to species of origin (Batty, Brambell, Hemmings & Oakley, 1954). It is apparently independent of molecular size, for the 7S equine diphtheria antitoxin reaches concentrations not more than 0.02 of those achieved by either 7S or 19S homologous antibody (Brambell, Hemmings & Henderson, 1951). The foetal cell is thus succeeding in

[1] See also Osorio & Myant, *Brit. med. Bull.* 1960, **16**, 159.–Ed.

FIG. 2. Relation Between the Degree of Iodination of Rabbit γ-Globulin and the Concentration Achieved in the Circulation, Three Hours After Feeding a Standard Dose to Young Mice



Hemmings (1960) by permission of Nature

fractionating a mixture of molecules according to a criterion which at least parallels their species antigenicity. The isolation of fragments of homologous γ-globulin by papain digestion (Porter, 1958) permitted the comparison of the whole molecule with its constituent parts, only one of which retains the antigenic specificity. That fraction alone enters at the rate of the whole molecule, the other fragments pass at about one-tenth this rate, despite the reduction of molecular size (Brambell *et al.* 1960). Similarly, pepsin refinement, which removes a large part of the equine, and presumably rabbit, antitoxin molecule, abolishing the species antigenicity but not the antitoxic activity, also reduces the rate of transmission of the molecule very drastically (Brambell *et al.* 1959).

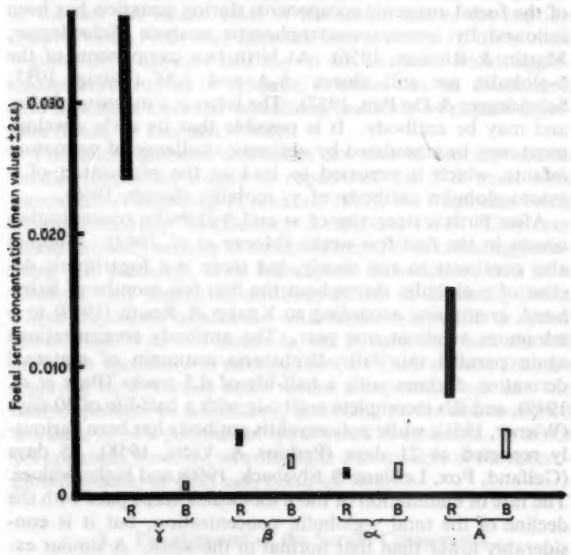
A much slighter change in the molecule, however, may also have a drastic effect on its ability to pass the endoderm cell. Iodination at the low level of 3 atoms per molecule of γ-globulin suffices to halve the rate of entry from the intestine to the blood stream; 5 atoms per molecule reduce the rate to approximately one-tenth (fig. 2) (Hemmings, 1960). Iodine reacts with protein by substitution in the benzene ring of the amino acid tyrosine. Evidently there is here the implication that quite a small number of tyrosine residues are vital to the normal entry of the molecule. When such "over-iodinated" protein is injected intravenously to adult animals, the circulating concentration declines more rapidly than it would were a lower proportion of iodine employed (Campbell, Cuthbertson, Matthews & McFarlane, 1956; McFarlane, 1956). This "screening" effect in the circulation seems to be less drastic than the selection which takes place during transport of the protein through the gut wall after feeding. The latter takes place much more rapidly, since the transfer is complete within a few hours after administration of the dose. There is here a readily available method of testing the biological integrity of protein preparations put up for in-vivo studies.

Holford (1930) and Schechtman & Abraham (1958) demonstrated quantitatively the passage of heterologous albumin into the foetal circulation of the rabbit, following intravenous administration to the mother. Fractions of homologous and heterologous (bovine) serum proteins, separated by zone electrophoresis and administered separately by the direct uterine route, are compared quantitatively in fig. 3. It is as though the rabbit endoderm cell were rather indifferent toward the α- and β-globulins. The requirement is low, and the foreign alternative is freely accepted. There is a degree of preference for albumin of its own kind, but a very firm distinction is drawn between the γ-globulins. Fully comparable data on species selection in other mammals are not available at present, but in both the monkey (Bangham, 1960) and the guinea-pig (Dancis & Shafran, 1958) it is clear that homologous albumin and γ-globulin enter at higher levels than α- and β-globulin.

In man, where the route of transfer still awaits critical identification, nature has fortunately designed the experiments by the provision of the iso-antibodies, and many of these are lucidly summarized by Race & Sanger (1958). Pedersen (1945) showed the ABO saline iso-agglutinins to be 19S components; they clearly do not reach the foetus save in rare cases. The molecules which do reach the foetus are 7S components and include the albumin-agglutinating, Coombs' sensitizing, and blocking antibodies. These are antigenically distinguishable from the macro-globulins, and Franklin & Kunkel (1958) used this elegant means to establish that the 19S components are absent from cord blood. Molecules such as typhoid O agglutinin, pollen reagins, diphtheria-precipitating antibody and cold auto-agglutinins are reported as macro-globulins, and the extensive literature of clinical observations which has been accumulated in much earlier work might well now be used retrospectively as a guide to the identity of other types of macro-globulin antibodies.

The intracellular mechanisms by which selection of proteins is achieved remain a fascinating puzzle. The endoderm cell

FIG. 3. Variation with Species of Origin and with Electrophoretic Mobility, of Serum Concentrations in the Foetal Blood of the Rabbit 24 Hours After ^{131}I Trace-Labelled Protein was Injected into the Lumen of the Uterus



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clearly behaves differently, according to very small differences of the protein entering it, and the selective transmission that results is very precise. The living membranes through which passive immunity is transmitted from mother to young provide a means of separation of serum protein components that is capable of experimental exploitation.

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IMMUNOLOGICAL TOLERANCE AND IMMUNOLOGICAL PARALYSIS

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The exposure of animals to antigenic stimuli before the maturation of the faculty of immunological response causes a specific weakening or suppression of reactivity during later life. Suppression of this type was first demonstrated experimentally in London in 1953, by Billingham, Brent & Medawar, and was termed by them "acquired immunological tolerance". Mice were shown to accept skin homografts after they had been inoculated during foetal life with cells from the donor. In the same year, in Prague, Hašek showed that chickens hatched from eggs that had been joined in parabiosis were unable to make antibodies against one another's blood (Hašek, 1953). These observations served to demonstrate experimentally a reaction which Owen (1945) in Wisconsin had already shown to occur naturally between dizygotic cattle twins sharing a synchorial placenta. The general features of tolerance were later worked out with tissue and erythrocyte antigens (Brent, 1958).

Tolerance can be acquired only during a limited period, which coincides roughly with foetal life. Sheep and guinea-pigs lose susceptibility before birth, as human beings probably do as well (Uhr, Dancis & Neumann, 1960); while mice, rabbits, and chickens remain susceptible for a few days after birth. After susceptibility has been lost, a brief "null period" follows, during which the animal cannot yet be immunized. Inert antigens can also be introduced into the embryo too early to induce tolerance, probably because they are eliminated before the future reactive tissue has differentiated. Another feature of tolerance is its exquisite specificity. For example, mice which have been rendered tolerant of homografts from one inbred strain can still reject grafts from other strains; and chickens tolerant of the erythrocytes of one individual turkey can react against erythrocytes from another individual. From this it follows that the stimulus for tolerance is an antigenic one. And, a third defining feature of tolerance, the site of action of this stimulus can be identified. Transplantation experiments with lymph-node cells have proved that the induction of tolerance involves changes in those cells that would normally respond by the formation of antibodies, i.e., cells which may be found in lymph nodes, the spleen, and elsewhere in the body. Mice of strain A were rendered tolerant of strain B, and inoculated with lymph-node cells from A strain donors that had been immunized against B strain antigens, or with cells from normal A strain donors. Both treatments caused the breakdown of tolerance, thus showing that reactive cells were previously lacking in the tolerant animal.

Although susceptibility to the induction of tolerance is lost, by definition, in adult life, antigens can still induce specific immunological unresponsiveness under certain special conditions. The treatments which do so include feeding with chemical allergens (the "Sulzberger-Chase effect"), overloading with polysaccharides or plasma proteins, transplantation of lymphoid cells into foreign hosts, and parabiosis. The problem is what relationship unresponsiveness of this type bears to tolerance in the classical sense. The antigens might conceivably act in an entirely different way: for example, the effects of overloading have been attributed to the presence in the body of an excess of antigen, which masks the production of antibody (Medawar, 1960). But even when satisfactory evidence can be adduced for "paralysis", in the sense used by Felton (1949) to denote a central failure of response, a different intracellular mechanism may operate. The principal arguments for distinguishing between tolerance and paralysis have been (i) that a larger dose of antigen is needed to induce paralysis, and (ii) that the maintenance of paralysis depends on the presence of antigen in the body, while this is not required for tolerance. These arguments can be examined only in contexts where the quantity of antigen can be measured. The present state of this problem is surveyed here.

1. Split Tolerance

Proliferation of the transplanted cells prevents most experiments with viable grafts from giving the information which is wanted about dosage and persistence of antigen. But the induction of tolerance by doubly antigenic cells provides a special case in which the need for persistence of antigen can be tested (Billingham & Brent, 1959; Billingham & Silvers, 1960). Baby female mice of one strain were inoculated with cells from males of another strain, for example, and later tested separately for tolerance of the homologous and Y antigens by means of skin grafts. Tolerance of the Y antigen lasted long after the grafts bearing the homologous antigen had been rejected, from which it was inferred that a state of split tolerance could exist in which tolerance extended to some but not all of the antigens of the original inoculum. It follows that the tolerant state could endure without the presence of antigen, or at least without the presence of the foreign antigenic cells.

2. Tolerance of Inert Antigens

The problem can be attacked more directly, using inert antigens. Those that have been most widely tested are the purified mammalian plasma proteins, particularly bovine serum albumin (BSA), bovine γ-globulin (BGG), human serum albumin (HSA), and human γ-globulin (HGG). All these proteins have been successfully used to induce tolerance in the rabbit (Hanan & Oyama, 1954; Cinader & Dubert, 1955; Dixon & Maurer, 1955; Smith & Bridges, 1958), BSA and BGG have been used with success in the mouse (Terres

& Hughes, 1959; Dresser, 1960), and BSA has been used with success in the chicken (Wolfe, Tempelis, Mueller & Reibel, 1957; Hirata & Schechtman, 1960). The most sensitive criterion of departure from complete tolerance of a protein antigen is provided by the rate of elimination of radioactively labelled antigen from the circulation; as judged by this criterion, rabbits and mice can be rendered completely tolerant. Chickens are more refractory, but a partial or complete suppression of precipitating antibody can be attained.

Tolerance of proteins provides the defining criteria for immunological tolerance of specificity and central failure. Tolerance of the major components of human plasma, for example, leaves rabbits still able to react against the minor components (Dixon & Maurer, 1955); and tolerance of a protein does not extend to diazo groups substituted in the same protein (Cinader & Dubert, 1955). Tolerance in rabbits can be abolished as expected by the transplantation of normal or immune lymph-node cells (Smith & Bridges, 1958; Weigle & Dixon, 1959). Central failure has not been demonstrated by the transplantation of cells in chickens; but the possible alternative mechanism of absorption of antibody by an excess of antigen can be excluded, since tracer measurements have shown that insufficient antigen is retained in the body (Hirata, Garvey & Campbell, 1960).

The dosage of protein needed to induce tolerance cannot be estimated without first considering the duration of the tolerant state. Observations on duration fall into two classes, according to whether the antigen was administered over a brief or a prolonged period. Short courses of administration can be considered as equivalent to a single injection—with BSA in the rabbit, at least (Dubert & Paraf, 1957). Brief treatments invariably produce a transient state of tolerance, extending over a period which depends on the dosage of antigen. The same type of result has been obtained in all the species that have been tested, although the quantitative relationship between dose and duration varies. In the rabbit, for example, Smith & Bridges (1958) found that an increase of dose from 0.1 mg. to 100 mg. of BSA resulted in an increase in the duration of tolerance from 50 to 150 days. From the half-life of BSA in the circulation, a figure of the order of 10^{10} molecules can be calculated for the quantity of antigen still present when tolerance is lost. The level of antigen in the circulation probably does not control the state of tolerance, however, because tolerance cannot be abolished by passive immunization with enough antibody to combine with the free antigen (Dresser, 1960; F. J. Dixon, personal communication). Yet the presence of antigen is required somewhere in the body, for tolerance can be prolonged indefinitely by periodic reinjections of antigen (Smith & Bridges, 1958; Dresser, 1960). More BSA is required, per unit body-weight, to induce a comparable degree of tolerance in mice, and more still in the chicken.

Does this dependence on the presence of antigen place tolerance of proteins in a separate category from the classical tolerance of tissues? Probably not, for a highly stable state of tolerance of protein can be induced by sufficiently prolonged treatment. Dixon & Maurer (1955) transfused rabbits at intervals with enough human or rabbit plasma proteins to replace the normal catabolic loss of native protein, over a period of 100 days from the day of birth. After this course of transfusions the rabbits remained unresponsive for 10–11 months, over the remaining period of observation. These results can be reconciled with those of Smith & Bridges (1958), and with split tolerance of tissue antigens, by assuming that the tolerant state gradually stabilizes; the stabilization could represent an accumulation of intracellular antigen, or a decline in the rate of cellular differentiation, according to which theory of tolerance is preferred.

Studies with erythrocyte antigens also reveal a gradual and progressive stabilization of the tolerant state. The rate of elimination of radioactively labelled erythrocytes can be used to detect slight immunity in chickens (Mitchison, 1959, and unpublished data). As judged by this test, a high degree of tolerance of homologous or turkey erythrocytes can be obtained by transfusion of eggs or newly hatched chickens. Normal blood confers long-lasting immunity, but this can be avoided by killing the leucocytes with radiation or heat treatment. Tolerance of treated blood can only be maintained by re-transfusion. The interval between elimination of antigen from the blood and the ensuing return of responsiveness can be measured, and has been found to increase gradually during the first months of life. The dependence on presence of antigen is much closer here than in previous work on the suppression of production of haemagglutinins (Hašek, 1956; Owen, 1956; Nossal, 1958), presumably because of the sensitivity of the test.

Neither plasma proteins nor erythrocytes can be regarded as totally foreign to the species in which they induce tolerance. Yet there is little reason to believe that more distant antigens would behave in a different way. Bussard (1957) succeeded in rendering rabbits tolerant of glucose-6-phosphatase extracted from yeast, by injecting as little as 2 mg. of the protein. A few successes and many failures have been obtained with microbial antigens (Cohn, 1957; Brent, 1958; Smith & Bridges, 1958; Weiss, 1958). There is no reason to believe that the microbial antigens are poor inducers on a molar basis, but they are too toxic to use in adequate quantities. The antigens tested by Cohn, for example, were injected into the embryo in quantities near the median lethal dose, yet even this amount represented less than 1% of the minimum dose of BSA needed to induce tolerance. Greater success can be expected as antigens free of contaminating toxin become available. With antigens as complex as the erythrocytes, the number of separate determinant groups must influence the ease with which tolerance can be induced. Hašek and his colleagues have found many examples of susceptibility to tolerance depending on phylogenetic relationship, which could be explained in this way (Hašek, 1956).

3. Immunological Paralysis

Turning now to immunological unresponsiveness acquired during adult life, one rarely finds specific central failure of response. The following four examples fall into this category.

a. The Sulzberger-Chase Effect

Delayed hypersensitivity can be acquired from contact with a variety of simple organic chemicals, such as picryl chloride or 2:4-dinitrochlorobenzene. The chemicals are thought to combine with dermal proteins to serve as antigens, and many of them also elicit the production of normal antibodies. Sulzberger (1929) discovered that the ability of guinea-pigs to become sensitized could be suppressed by prior intravenous injection of the chemical, but his experiment could not subsequently be repeated. A more repeatable method of suppression was discovered by Chase (1946), feeding the chemical.

Suppression produced in this way applies only to the chemical which was fed, and to cross-reactive compounds of similar structure. Ability to produce antibodies is also suppressed. A central failure of response has been brought about, for Chase & Battisto (1959) could confer a transient state of sensitivity of the animals showing such suppression, by transplanting reactive cells from sensitized donors.

This ability of guinea-pigs remains suppressed for many months, but not permanently. Large reserves of the chemicals cannot be detected in the tissues over this time, either by autoradiography or by the rate of elimination of passively administered antibody (Chase & Battisto, 1959). But there is nothing to suggest that the chemicals are completely eliminated, and indeed their ability to combine with cell proteins would suggest that some antigen could be retained.

b. Polysaccharide Paralysis

The injection of a large amount of pneumococcal polysaccharide (0.5 mg.) renders mice unresponsive, so that a small dose (0.0005 mg.) cannot subsequently protect them against infection with pneumococci (Felton, Kauffmann, Prescott & Ottinger, 1955). A similar state of unresponsiveness can be produced by excessive doses of the capsular material of *Klebsiella* (Orskov, 1956). The unresponsiveness is highly specific: cross-suppression occurs only with SIII against SVIII, in conformity with their chemical relationship (Stark, 1959). The outstanding peculiarity of the pneumococcal polysaccharide antigens is their exemption from normal catabolism in the body (Felton, 1949). Felton believed that this enabled the antigen to occupy some key site in the immunologically reactive cell, where it could block the formation of antibody. Doubts about this interpretation arose when Dixon, Maurer & Weigle (1955) showed that the antigen fixed in tissue could remove labelled specific antibody from the circulation. The hypothesis of antibody absorption is advocated by Stark (1955, 1959), on the ground that the polysaccharide is retained in tissue but gradually loses its antigenicity there, in the way that would be expected if it were suffering damage from repeated interactions with antibody.

Histological evidence has recently confirmed Felton's original hypothesis of central failure. Gitlin, Monckeberg & Craig (1958) could find no evidence of immunological reaction in the lymph nodes of mice which had received large doses of pneumococcal polysaccharide, although a weak reaction could be detected after immunization with small doses. Sercarz & Coons (1959) demonstrated the presence of intracellular antibody in the reactive tissue of immunized mice by fluorescent staining, but were unable to do so in mice which had been paralysed. The tissues of mice which had been paralysed by repeated injections of BSA were also examined, and likewise found to be free of antibody-containing cells.

c. Protein Overloading

Dixon & Maurer (1955) examined the effect on adult rabbits of the same course of massive and repeated transfusions which had been used to induce tolerance. After six weeks or longer of transfusions, the rate of elimination of antigen from the circulation was equal to that found in the tolerant animals. The rate remained unaltered even when the level of antigen fell to trace levels, thus showing that the antigen could not have masked the production of antibody. The specific nature of the suppression was demonstrated by production of antibodies against the minor components of the transfused plasma. The application of fluorescent staining by Sercarz and Coons has confirmed the absence of antibody production, as mentioned above.

The rabbits of Dixon and Maurer soon developed serum sickness, which was attributed to the reaction of antibodies directed against the minor components of the transfused plasma. An old experiment of Glenny & Hopkins (1924) casts doubt on this interpretation. A rabbit was repeatedly injected with antitoxic serum from a horse; the horse globulin thus served as an antigen, which could be titrated by its antitoxic value. The level of antigen in the rabbit circulation rose at first, then fell as antibody production commenced, and finally rose again as the animal entered a state of paralysis; slow and constant elimination was observed after the injections stopped. It appears then that rabbits react initially, but can be overwhelmed by a continuous influx of protein. Mice, on the other hand, react weakly or not at all against an intravenous injection of BGG, unless they have been "activated" by an adjuvant (Dresser, 1960). It is presumably for this reason that they are extremely susceptible to the induction of paralysis; indeed, Dresser finds that the paralytic dose is no larger than the dose required for the induction of tolerance, per unit body-weight.

Paralysis cannot be established quite as firmly as tolerance in the rabbit. Dixon and Maurer found that their paralysed rabbits eventually became reactive again, although those which had been rendered tolerant did not. This is no more than a quantitative difference, for the antigens were the same as those which have now been shown to be capable of inducing a transient state of tolerance.

d. Graft-versus-Host Reactions and Parabiosis

That grafts can react against their hosts is something which could not be expected if an overwhelming dose of antigen would necessarily induce paralysis. Evidence of paralysis of grafted cells by host antigens has recently been reported by Simonsen (1960), although his results could not be repeated in another strain combination by Siskind, Leonard & Thomas (1960). Spleen cells were transplanted into F_1 hybrids, where a brief reaction took place against the surrounding tissue, although the donor cells remained in the spleen of the host for a longer period. The immunological capabilities of these residual cells were tested by further transplantation: although they could react against other antigens, they had lost the capacity to react against the antigens of their first host. Parabiosis appears to have the same effect on the cells of inbred mice which have been joined with an F_1 hybrid partner (Rubin, 1959; Martinez, Shapiro & Good, 1960). Skin grafts of the partner's antigenic constitution may survive indefinitely after the union has been broken, but only if the union has already been maintained for at least 60 days.

To summarize, adult animals can be rendered immunologically unresponsive by a variety of treatments. Those mentioned appear to bring about central failure of response, and so are essentially similar to the induction of tolerance in the immature animal. The problem is what these treatments have in common to distinguish them from the normal course of events which leads to immunity after exposure to antigen. The dosage of antigen can hardly be critical, for much more protein is needed than chemical allergen or polysaccharide. May it not be that prolonged exposure to antigen is what is

required? If that is the case, large quantities of protein would be needed to maintain the presence of antigen in the body in the face of a powerful immune reaction, and the same would apply to parabiosis or a foreign environment in the case of the transplantation antigens. The need would be absent in situations in which an antigen can be administered in a form, or by a route, such that no reaction is called forth. This applies to the chemical allergens, and to a lesser extent to poor antigens like the highly purified polysaccharides. The need is further reduced in these cases by the long life of the antigen after fixation in tissue. The fact that protein antigens can paralyse mice more easily than rabbits lends further support to this view.

This view implies that immature animals can be rendered tolerant simply because they cannot react against antigens. In the immature animal the antigen is able to persist, and so exert its suppressive or inhibitory effect on the maturing reactive tissue, without provoking concomitant immunity. All antigens, in fact, can behave in the immature animal in the same way as do the orally administered allergens in the adult. From this point of view the null period is an artifact; the period of susceptibility to the induction of tolerance terminates when the animal can make enough antibody to be able to eliminate the antigen.

4. Theories of Tolerance

Events at the cellular level are still a matter of speculation (Burnet, 1957; Lederberg, 1959; Medawar, 1960; Mitchison, 1959). The question whether the immunologically competent cells make up a homogeneous population has been much discussed. The homogeneous model treats all the cells as potentially capable of being rendered tolerant (and perhaps killed in the process) at any time of life. Adults differ from the immature animal simply in the quantity of antigen needed to bring about the transformation: less is needed in the foetus, either because the cells themselves need less for the transformation, or because the foetal environment facilitates the access of antigen. During the induction of paralysis, the same cells undergo inhibition as could otherwise produce antibody. The heterogeneous model, on the other hand, assumes that non-reactive stem cells pass through a transitional stage, at which they can be rendered tolerant, before differentiating into mature reactive cells. The immature animal contains stem cells alone, so that the introduction of an antigen at this stage can block the recruitment of mature cells. Differentiation continues to take place during adult life, so that the presence of antigen is required to maintain the block and hence the tolerant state. The fact that paralysis can be induced in the adult implies that the life-span of the mature cell is limited. During prolonged exposure to antigen the recruitment of new mature cells is blocked, and the existing stock gradually depleted.

The part which chronic antigenic stimulation appears to take in paralysis lends support to the heterogeneous model. But many of its predictions remain to be tested. It predicts, for example, that normal immunization should be accompanied by the production of tolerant cells; that a lag period should precede the inception of paralysis after a single injection of antigen; and that the tolerance-inducing and tolerance-maintaining dosage of antigen should be identical. The heterogeneity which it predicts also remains to be related to the morphological differentiation of lymphoid tissue.

These theories of population dynamics leave the problem of suppression in the individual cell still unanswered. The cell could be rendered unresponsive in three alternative ways:

i. Cell death. According to the clonal selection theory, mutable stem cells give rise to clones of cells producing different specific antibodies, and tolerance is acquired through the destruction of clones by the action of antigen. Lederberg suggests that the transition from stem cells to mature clones takes place repeatedly throughout adult life. During the transition the cell is hypersensitive and can be killed by interaction with antigen. This is a version of the heterogeneous population model, though not the only one.

ii. Inhibition of antibody formation. The tolerant cell might catabolize antigens faster than its sensitive counterpart, as Humphrey (1956) has suggested. By so doing it could prevent the formation of antibody, if the presence of antigen is needed for formation to take place. Developing this idea, Medawar has suggested that antigens elicit tolerance by inducing the formation of specific degradative enzymes. As in theory (*i*), antigen plays an elective role, and an individual cell would be expected to be either fully tolerant or fully reactive to a single antigen. The fact that states of partial tolerance are so often encountered raises a difficulty. Partial tolerance of single cells has not yet been demonstrated. But it seems likely that they occur in animals which are partially tolerant of purified antigens (Terres & Hughes, 1959; Dresser, 1960).

iii. Destruction of formed antibody. Partial tolerance can be easily accounted for, but the absence of histological evidence of antibody formation during tolerance, the slow elimination of passive antibody, and the activity of transplanted lymphoid cells are more difficult to reconcile with this mechanism. Yet absorption of antibody by antigen sequestered within the reactive cell remains a possibility.

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TRANSPORT MECHANISMS IN THE FOETUS

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1. Definitions of Transport Mechanisms

The term "transport" in a physiological context usually implies the movement of ions, substrates, or the products of metabolism, which may be divided into processes involving firstly transport in bulk, such as the circulation, lymph flow, the propulsion of intestinal contents and renal tubular flow, and secondly transport across individual cell membranes, epithelia, or similar structures separating various physiological compartments.

The circulation and other forms of bulk transfer will not be considered here, but it should be realized that several complex physiological processes, e.g., the working of the kidney, involve a specialized morphology in which both forms of transport are interrelated. The way in which these are integrated in the formation of hypertonic urine (Wirz, Hargitay & Kuhn, 1951) is a good example. Possible integrations in foetal physiology will be considered later.

a. Diffusion, Facilitated Diffusion and Active Transport

It is now generally agreed that the passage of substances into and out from the cytoplasm of cells can be classified in three ways. Danielli (1954) has defined these as diffusion, facilitated diffusion and active transport.

Diffusion is a transfer down a chemical potential gradient in which the kinetics obey Fick's Law, and the rates are in accordance with predictions based on the physicochemical properties of the substance.

Facilitated diffusion is not so easy to define, but has a number of recognizable characteristics, the more important being (i) a faster rate of transfer than would be predicted on physicochemical grounds; (ii) kinetics which deviate from Fick's Law and usually show saturation at high concentrations; (iii) competition for the transfer mechanism by related compounds and (iv) possibly specific inhibitors which are competitive in nature. As with diffusion, the transfer is usually down a chemical potential gradient, and the simplest working hypothesis is that the substance combines chemically with a component of the cell membrane which possibly acts as a carrier taking the substance across the membrane in the form of a complex. The existence of such mechanisms has been proved chiefly for those substances which are insoluble in the lipid membrane of cells. These are sugars, amino acids and salts, but many other molecules are probably involved as well.

Active transport involves the transfer of molecules across membranes by the expenditure of metabolic energy, and the molecular species may be transferred against an electrochemical gradient. Thus, whenever an ion or molecule is known to be able to pass across a membrane and is found to be in a steady state which does not correspond to electrochemical equilibrium, there is a priori evidence for an active transport. The number of active transports of a primary kind is probably very few, and only that for sodium and potassium (see Harris, 1954; Ussing, 1954; Hodgkin & Keynes, 1955) is well authenticated in animal tissues. This primary process for sodium and potassium, however, is postulated to be linked, and again the simplest working hypothesis is to suppose the existence of membrane carriers whose affinities towards Na and K change, at opposite sides of the cell membrane, in relation to certain metabolic activities (Caldwell, Hodgkin, Keynes & Shaw, 1960).

b. The Origin of Membrane Potentials

It is important to realize that the active transport across a homogeneous cell membrane can set up a concentration difference only between the cell interior and its immediate environment. Where the substance is an ion with a capacity to penetrate the cell membrane passively, a membrane potential is set up. In the case of certain cells permeable to K^+, this potential approximates to that predicted by the Nernst equation:

$$E = 60 \log_{e} \frac{[K_i^+]}{[K_o^+]}$$

where E = electrical potential in millivolts, and

$[K_i^+]$ and $[K_o^+]$ = potassium ion concentration inside and outside the cell, respectively.

However, for a potential to be set up across an epithelial or similar membrane the active transport must be asymmetrical in regard to the borders of the epithelial cells facing in opposite directions. For example, the origin of the potential across the frog's skin as visualized by Johnsen & Ussing (1956) involves (i) the outer cell borders being passively permeable to Na^+ ions while being relatively impermeable to K^+ ions; (ii) the inner cell borders being passively permeable to K^+ ions but relatively impermeable to Na^+ ions (passively); (iii) an active mechanism on the inner border only which transfers Na^+ out of the cell and is linked to an active uptake of K^+.

This active mechanism maintains the intracellular sodium concentration low and the potassium high. The passive transfers result in a sodium diffusion potential (E_{Na}) across the outer border and a potassium diffusion potential (E_K) across the inner border. These are of the same sign, and the total potential across the skin is the sum of these two: $E = E_{Na} + E_K$. Chloride is induced to follow the sodium because of this electrical potential.

Methods of study used by Ussing and his colleagues have been applied by Crawford & McCance (1960) to the chorio-allantoic membrane of the pig, in which a similar mechanism for transferring sodium ions from the foetal to the maternal side exists.

Since the development of potentials across epithelia thus depends on cell differentiation, the study of membrane phenomena in foetal development might be expected to yield additional information on the underlying mechanisms as well as a better understanding of foetal physiology. However, this field has received little attention, although a number of preliminary surveys have been made. These have taken the form either of comparison of foetal cell permeabilities with corresponding adult cells, studies on developing tissues (e.g., nerve-muscle preparations or kidney slices), or studies of the permeability of the placenta and of the amniotic and allantoic membranes.

2. Comparisons Between Maternal and Foetal Red Cells

a. Permeability to Water

The water permeability of red cells can be indirectly measured by following the rate of volume increase in hypotonic salines. Using a photoelectric method, Widdas (1951) found that red cells of foetal sheep had a lower water permeability than red cells of the adult sheep. Sjölin (1954) made a similar survey of red cells from cord blood and human foetuses and extended the study to osmotic and mechanical fragility. He confirmed that water permeability is lower in red cells from human foetuses and new-born. Both authors attributed their findings to a difference in membrane properties. On this interpretation there is also a progressive change with foetal age (Widdas, 1951).

b. Permeability to Sugar

Human adult red cells possess a facilitated transfer mechanism for glucose (Lefevre, 1948; Widdas, 1954a; Wilbrandt, 1954), which is capable of passing glucose into the cell at a rate some two hundred times the rate of glucose utilization. In consequence, in man the cell water rapidly equilibrates with the plasma glucose, but the same is not true of other species (Somogyi, 1933). In those species where this equilibration is incomplete the transfer mechanism for glucose is either not detectable or is present to a very small degree, and Morgan, Kalman, Post & Park (1955), who estimated the transfer system of rabbit red cells, found it to be about one-thousandth that of human red cells.

The red cells of foetal blood in a number of species equilibrate much more completely than those of the adult animal. Thus Andreen-Svedberg (1933) found in calf red cells a glucose concentration which in mg./100 ml. of red cells was 59% of the concentration of the supernatant plasma. This is termed a cell-plasma glucose concentration ratio of 59%. For piglets it was 42%. Hitchcock (1949) found a ratio of 73–84% for fructose in the foetal sheep. This type of distributional study was extended by Goodwin (1956) to the foal, pig, lamb, calf, guinea-pig and rabbit.

By following the kinetics of glucose penetration into foetal cells, and also that of sorbose, which is competitively inhibited when glucose is present, Widdas (1955) was able to show that glucose penetrates foetal erythrocytes of sheep, deer, pig, guinea-pig and rabbit by a facilitated transfer similar to that in red cells from human adults. There are also differences between adult and foetal red cells in regard to their permeability to glycerol and erythritol (Bowyer & Widdas, 1955) and to vitamin C (Räihä, 1958). In these respects, foetal red cells have abilities which are lost in the adult.

These results reinforce the view that there are differences in the membrane properties between the foetal and the adult state.

c. Ionic Permeability and Distribution

Ionic permeabilities of foetal red cells have received scarce attention. Sjölin (1954) compared the K^+ loss of cold-stored human red cells from cord blood and found this to be higher than in adult cells. Foetal cells are generally less resistant to cold storage than adult cells.

Indirect evidence of permeabilities may be suggested by ionic distribution studies. Differences are more noticeable in some of the mammals which (as adults) have red cells with low potassium concentrations. Thus red cells of the ox have low K^+, but Wise, Caldwell, Parrish, Flipse & Hughes (1947) found the red cells of young calves to have a high concentration of K^+. In the sheep, red cells tend to be of two or more types (Kerr, 1937; Evans, 1954, 1957; Widdas, 1954b) in regard to potassium content. The potassium content of foetal red cells of the Welsh breed was shown to be high irrespective of the type of cells of the parent (Widdas, 1954b).

Cationic flux measurements on foetal cells have not so far been reported, but Tosteson (personal communication) has examined the fluxes in cells from lamb's blood as their properties change to the adult type during the first six weeks after birth.

The various studies thus show that foetal red cells are equipped with special transfer mechanisms for sugars and for accumulating potassium at least as efficient as (and in many mammals more efficient than) the corresponding cells in the adult. If this could be extrapolated to other tissues it would suggest that any deficiency in a physiological function concerning membrane phenomena may lie in immature differentiation or morphological re-arrangement rather than in the cells as such. This seems to be the case in the foetal kidney's inability to concentrate urine during early gestation (Alexander, Nixon, Widdas & Wohlzogen, 1958).

3. Placental Permeability Studies

Placental transport has been extensively studied and a recent review by Hagerman & Villee (1960) is available, but these authors distinguish only between simple diffusion and active transport. Certain aspects of mineral transport are discussed elsewhere in this Bulletin by McCance & Widdowson (1961), while Hemmings & Brambell (1961) deal with aspects of protein transfer across the placenta. Topics will therefore be selected which illustrate problems involving transport mechanisms and the maintenance of osmotic equilibrium between maternal and foetal circulations.

a. Permeability to Water

Using isotopic methods, Gellhorn & Flexner (1942) showed that water crossed the placenta 150–500 times more rapidly than the net uptake of water by guinea-pig foetuses. Plentl (1958) demonstrated a similar rapid turnover of water in the human foetus, and it must be assumed that osmotic equilibration across the placenta occurs (Meschia, Battaglia & Barron, 1957). By artificially changing the maternal plasma osmotic

pressure in the rabbit, Dancis, Worth & Schneidau (1957) obtained equilibration by the foetus within about 80 minutes. McGaughey, Jones, Talbert & Anslow (1958), by analogy with the rates of equilibration of antipyrine (mol.wt. 180), deduced that water (mol.wt. 18) must achieve full equilibrium as the blood passes once through the placenta.

b. Permeability to Sugars

Although Anselmino (1929) considered that non-electrolytes of molecular weight under 350 might cross the placenta by physical diffusion, this seemed unlikely in ungulates (Huggett, Warren & Warren, 1951) where the foetal blood contains a high concentration of fructose. On a basis purely of molecular weight, fructose should be expected to cross the placenta as fast as glucose, but this is not so even in non-ungulate placentas. Karvonen & Räihä (1954) showed a ratio of over 10:1 in favour of glucose in the guinea-pig, and a similar ratio was shown in man by Holmberg, Kaplan, Karvonen, Lind & Malm (1956) and by Chinard, Danesino, Hartmann, Huggett, Paul & Reynolds (1956). Differential permeability towards aldoses and ketoses in the rabbit placenta has been shown by Davies (1957), and most workers agree that placental transfer of sugars is either an active process or one of facilitated transfer as proposed by Widdas (1952). There is no evidence that glucose is moved up a concentration gradient in any of the transfer studies so far, and the fact that reduced oxygen tension is without effect (Karvonen, Laamanen & Räihä, 1956) is also suggestive that active transport is not involved. The facilitated transfer of glucose across red-cell membranes, discussed above, has many of the features observed in placental transfer. In the ungulates there is in addition the synthesis of fructose on the foetal side. This aspect is being considered elsewhere in this Bulletin (Huggett, 1961) but the point should be made that the concentration of the combined sugars on the foetal side may exceed that on the maternal side. However, this accumulation does not indicate active transport at the placental membrane.

In this connexion it may be pointed out that vitamin C occurs in higher concentration in foetal blood than in maternal blood. Räihä (1958) has shown that the placenta is similar to red-cell membranes in that dehydroascorbic acid rapidly crosses both, whereas L-ascorbic acid does not. He attributes this to the fact that dehydroascorbic acid has a lactone ring and is un-ionized, but L-ascorbic acid behaves like an electrolyte. Räihä also advanced evidence to suggest that reduction of dehydroascorbic acid (which passed across the placenta down its own concentration gradient) accounts for the high concentration of vitamin C (L-ascorbic acid) in the foetus. Thus, although accumulation is the result of the differential permeability of the two forms, it does not involve active transport by the membrane.

c. Permeability to Amino Acids

It was demonstrated by Van Slyke & Meyer (1913) that the concentration of free amino acids is higher in foetal than in maternal blood. This has been confirmed by other workers (Christensen & Streicher, 1948; Clemetson & Churchman, 1954), and Setnikar (1957) has made quantitative measurements of the rate of transfer of amino acids across the placenta of pregnant goats in which the foetal concentration of amino acids is about double that of the maternal. Christensen & Streicher (1948) have drawn attention to the functional similarity of cell and placental membranes in their ability to accumulate amino acids against a concentration gradient, but there is still no clear evidence of a membrane active transfer as opposed to an accumulation mechanism akin to that for vitamin C. That amino acids do not cross the placenta by simple diffusion is supported by the preferential permeability of the L-isomer relative to the D-isomer (Page, Glendening, Margolis & Harper, 1957) and this strongly suggests at least a facilitated transfer of the type postulated for hexoses.

d. Permeability to Salts

The permeability of placentas to common cations such as Na^+, K^+ and Ca^+ has been extensively studied. Tracer techniques were applied to this problem by Flexner and his colleagues in animals and man.

In the case of the human placenta, Flexner, Cowie, Hellman, Wilde & Vosburgh (1948) showed that the placental exchange of radioactive sodium indicated a permeability 160 times greater than the sodium accumulation at 12 weeks' gestation and over 1,100 times greater at 40 weeks' gestation. Cox & Chalmers (1953) obtained a ratio of 450 near term. McGaughey *et al.* (1958), by comparing the rate of equilibration of antipyrine with that of sodium, conclude that the placenta offers considerable hindrance to the movement of charged particles, and it must be agreed that the equilibration of water (osmotic) will occur much more readily than that of salts.

For the literature on the transfer of iron, iodine and other trace minerals, reference may be made to the review by Hagerman & Villee (1960) and to the paper in this Bulletin by McCance & Widdowson (1961). Although some of these ions are clearly at a higher concentration in the foetal than in the maternal circulation, their contribution to the foetal osmolarity can be neglected. Iron, for instance, is probably bound to protein, and a placental iron complex (possibly ferritin) is involved (Nylander, 1953).

The accumulation of calcium in the foetus (Needham, 1931) has also been the subject of study by isotopic techniques: see, for example, Wasserman, Comar, Nold & Lengemann (1957). The whole subject of studies with radioactive calcium was reviewed by Comar (1956) who was led to the conclusion that active transport took place, on the basis of "against the gradient" transfer. Comar also pointed out that placentas are characterized by high arginine concentrations which are also effective in promoting calcium absorption from the digestive tract.

It is only right to point out that the evidence for active transfer in these cases is based on accumulation against a concentration gradient, and no evidence of transfer against an electrochemical gradient has been demonstrated experimentally. Further, no clear distinction is made between an accumulation mechanism and an active transport by the membrane.

4. Intra-Uterine Potentials

The determination of electrochemical gradients requires the simultaneous determination of concentrations and transplacental electrical potentials. Experimental determinations of the latter have been very scarce. Meschia, Wolkoff & Barron (1958) measured the difference in electrical potential across the placenta of goats. They found potentials between maternal and foetal blood streams of between 25 and 133 mv, the foetal circulation being negative. The potential was higher

in early gestation and lower towards term. It was suggested that the chorion was the site of origin of this potential.

Widdas (unpublished observations) measured potentials in the amniotic fluid, allantoic fluid and foetal blood of three sheep relative to a reference electrode in the peritoneal cavity of the ewe. The results in Table I show that a transplacental potential of some 30 mv existed (the foetal circulation being negative). In earlier preliminary experiments potentials of 30–40 mv had been obtained between the amniotic cavity and maternal peritoneum in the pregnant cat.

TABLE I. Intra-Uterine Potentials in the Sheep

Gestation age (days)	Amniotic sac (mV)	Allantoic sac (mV)	Foetal blood stream (mV)
127	72–85	82–87	—
140	64–70	50–60	24–28
140	50–52	70	35–40

Table I shows the ranges of electrical potential in millivolts (mV) between the maternal peritoneal space and three sites in the conceptus. All measurements were negative with respect to the mother's peritoneum.

Measurements of these electrical potentials are given significance by the findings of Crawford & McCance (1960) that the chorio-allantoic membrane (of the pig) actively transfers sodium ions from the foetal to maternal surface. This would make the whole contents of the sac negative relative to the mother, and (assuming similar activity occurs in the goat, sheep and cat) could account for the observed potentials.

The presence of an electrical potential of 30 mv or more across the placental barrier (the foetal side being negative) would be against inferring an active sodium transfer from mother to foetus in the placenta but would produce an electrochemical gradient in that direction which would promote the passive movement of all cations towards the foetus. For instance, electrochemical equilibrium would not be attained until the foetal–maternal concentration ratio for monovalent cations was threefold and that for divalent cations tenfold. This means that calcium could be passively accumulated (by reason of this potential difference) without postulating a specific active mechanism for the calcium ion.

5. Integration of Transport Mechanisms in Foetal Growth and Uterine Expansion

One of the key problems in foetal physiology is the maintenance of an adequate fluid volume in the foetal and placental vasculature and in the amniotic and allantoic sacs, to permit continual growth of the foetus and expansion of the uterus.

Treating the placental barrier as equivalent to capillary membranes across which an equilibrium might be set up in accordance with Starling's hypothesis, Barcroft (1946) saw that the foetus would be at a disadvantage because of the lower plasma concentration of protein there (and hence lower colloid osmotic pressure) than in the maternal circulation. More recent work, that of Barboriak, Meschia, Barron & Cowgill (1958), confirms this view. Compensatory hydrostatic pressures seemed unlikely, since a large hydrostatic pressure difference between maternal and foetal circulations in the placenta could lead to collapse of foetal vessels. It is now clear from the newer data on placental permeability that the placental barrier cannot be treated as equivalent to the capillary wall.

Consequently, it is probable that the non-colloid osmotic pressure is more important than the colloid, but it is still too early to give a complete answer to this problem. In an attempt at synthesis one can see a number of mechanisms which are in favour of the foetus in this respect.

i. Gases. In regard to gaseous exchange, Barron and his co-workers have found partial pressure differences for O_2 and CO_2 across the placenta of the order of 15–20 mm. Hg. In the case of oxygen the gradient is in favour of the mother, but since the dissolved oxygen is negligible, compared with the oxygen carried by haemoglobin, this is not as unfavourable osmotically as it might seem. Indeed, as is well known, oxidized haemoglobin has a greater base-binding capacity than reduced haemoglobin, and this factor works to the osmotic advantage of the foetus. On the other hand, Blechner, Meschia & Barron (1960) have estimated that there is an increase of 0.24 m-mole/l. of dissolved CO_2 in foetal plasma as compared with maternal plasma. Thus the net osmotic consequences of the gradients necessary for gas transfers are in favour of the foetus.

ii. Non-electrolytes. In carbohydrate transfer the existence of a concentration gradient from mother to foetus is osmotically unfavourable, but in ungulates this is more than made good by the fructose, as pointed out by Barcroft (1946). A gradient of 30 mg./100 ml. is about 1.7 m-osmole/l., and in non-ungulates a difference of this order would have to be made good in other ways, e.g. by a foetal–maternal gradient of products of metabolism. It may be noted that even in non-ungulates inositol occurs in a higher concentration in foetal than maternal plasma (Nixon, 1952). Amino acids, vitamin C and other substances accumulate in amounts which are of little osmotic significance in themselves, but are all osmotically additive and favour the foetal circulation. On the other hand anabolic processes, such as incorporation of amino acids into proteins, tend in the opposite direction (Stanier, 1960b).

iii. Salts. However, the above considerations are probably of minor importance. As with the maintenance of interstitial fluid, the major factor is likely to be the control of the salt content and particularly that of the cation sodium. There can now be seen to be at least two factors at work which must assist in the retention of salt in the foetal circulation and the maintenance of osmotic equilibrium across the placenta. These are firstly the production by the kidney of considerable volumes of hypotonic urine (Alexander *et al.* 1958; Stanier, 1960a), and secondly the active transport of sodium by the foetal membranes, generating a transplacental potential in favour of a passive sodium flow across the placenta from mother to foetus. Meschia *et al.* (1958) also consider that electro-osmosis might occur. This could be a third factor.

The production of hypotonic urine enriches the plasma sodium in the renal vein and in turn raises the osmotic pressure in the umbilical artery. The magnitude of the effect in the sheep can be calculated from data on placental blood flow, as reported by Cooper, Greenfield & Huggett (1949), in conjunction with measurements of urine flow and osmotic pressure by Alexander *et al.* (1958). It is not large but it is greater in young foetuses, falling from about 25 mm. Hg at 60 days' gestation to 1 mm. Hg near term.

The foetal urine passes into the allantoic sac and possibly

later into the amniotic sac. The chorio-allantoic membrane further reduces the salt content by the active transfer of Na outwards to the maternal surface (Crawford & McCance, 1960), but as the sodium and chloride contents of the fluid fall below that of urine (Alexander *et al.* 1958), it appears that the sacs are relatively impermeable to water (see also Garby, 1957). Thus the active transport which sets up the membrane potential is at the expense of that sodium which (because of the relative impermeability of the sacs to water) has largely lost its osmotic importance to the foetus.

These factors may be influenced in some placentas by the counterflow of the respective blood streams (Barcroft & Barron, 1946; Noer, 1946); but owing to the rapid movement of water across the placenta it is likely that the osmotic gradient between maternal and foetal plasma will be very small. This is suggested by the results of Meschia *et al.* (1957), who found that (if proper precautions are taken) the osmolarity of foetal plasma is equal to or slightly less than maternal plasma.

In addition to these factors, the underlying mechanisms concerned with the formation of amniotic fluid and its reabsorption following swallowing (Garby, 1957; Plentl, 1959) must also be integrated in this problem—which still remains a subject for experimental investigation.

6. Conclusion

Investigations of foetal and maternal red cells have shown that the former possess mechanisms for facilitated and active transfer at least as potent as the latter. The suggestion that the placental membrane is functionally similar to that of a cell (Christensen & Streicher, 1948) is well borne out by studies on the permeability of sugars, vitamin C and amino acids. Thus, facilitated transfers by the placenta have received considerable attention.

The critical study of active transport and membrane potentials in relation to foetal physiology has scarcely begun, but it can already be seen that active transport of sodium in the renal tubules and the chorio-allantoic membrane may indirectly make a significant contribution to wider aspects of foetal physiology, both in regard to osmotic equilibrium and accumulation mechanisms. The study of transport mechanisms in relation to foetal physiology is clearly a field from which we may expect to gain important ideas.

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THE FOETAL KIDNEY

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Interest in renal function during foetal life was apparent in the writings of many early observers (see Needham, 1931). The mechanism of the formation of urine and its relationship to the amniotic fluid and allantoic fluid had, however, to await the commencement of anatomical and physiological investigations in the nineteenth century.

Investigation of renal function in man has been limited to the analysis of bladder urine samples obtained from aborted foetuses and to the collection of urine from premature infants during the first few days of life. A more extensive investigation of renal function during foetal life has been derived from experimental animals. Recently, techniques have been developed which extend the scope of the investigations by permitting rates of urine flow to be measured and, hence, plasma clearances to be determined (Alexander, Nixon, Widdas & Wohlzogen, 1958b; Boylan, Colbourn & McCance, 1958).

1. Anatomical Considerations

a. Structure of the Kidney in Foetal Life

The renal system in many vertebrates develops successively with overlapping through three stages: pro-, meso- and metanephros. In man, the pronephros is vestigial and is replaced in about 4-mm. embryos by the mesonephros. This is a larger organ situated posterior to the pronephros, containing a more complex system of functional tubules derived from the nephrogenic cord and opening into the mesonephric duct. The organ attains its maximum development between the fourth and ninth weeks. From the caudal end of the mesonephric duct develop the ureter, renal pelvis and collecting duct, while from the nephrogenic cord stems the tubular system. The union between collecting duct and one end of the tubule, and the formation of a glomerulus within a blind indentation of the other end of the tubule, result in the nephron of the metanephric or permanent kidney. Development of the glomerulus from a solid sphere of mesodermal cells has been described in the rabbit, guinea-pig and sheep (Lewis, 1958) and man (Kurtz, 1958). Histological investigations on the meso- and metanephros of the foetal rabbit, using the light microscope and the electron microscope, have been reported by Leeson & Baxter (1957) and Leeson (1959, 1960). The mesonephric kidney persists for a varying period of time in different mammalian species. For example, in the pig degeneration occurs in the first half of gestation, whereas in the rabbit it takes place in the second half.

In this review attention will be focused mainly upon the metanephric kidney.

In the foetus, the kidney makes up a larger proportion of the total body-weight than it does in the adult. Thus, in the foetal sheep the percentage of the body-weight due to the kidney steadily declines from 1.45% at a foetal age of 65 days to 0.5–0.6% at term; in the adult the average is 0.36%. In the immediate postnatal period the percentage may rise to 0.95% owing to relative dehydration.

Histological studies had suggested that the reabsorptive elements of the nephron, but not the glomerulus, are well developed by term. In man, Gruenwald & Popper (1940) suggested that the tall columnar epithelium of the glomerular capsule would impair filtration in foetal life. Glomerular filtration was said to occur at birth by the sudden rupture of the epithelial layer covering the vessels of the glomerulus. However, in the sheep high rates of urine flow are observed (see section 2), and the clearance of dextran fractions of known molecular size in the foetal sheep failed to indicate a change in the glomerular permeability with age (Alexander and Nixon, to be published).

Three phases in the maturation of the glomeruli have been identified histologically by Macdonald & Emery (1959): first, a period up to about the 36th week of gestation during which new glomeruli are formed; second, a period extending until the 3rd–5th year of age where the glomeruli, although mainly immature, are present in numbers similar to those in adults; finally, a period extending from the 3rd to the 12th year, during which full maturation of the glomeruli occurs. It would appear that the production of new glomeruli is dependent primarily upon body-weight, and only secondarily upon gestational age, and that in the premature infant glomerular production continues after birth (Potter & Thierstein, 1943).

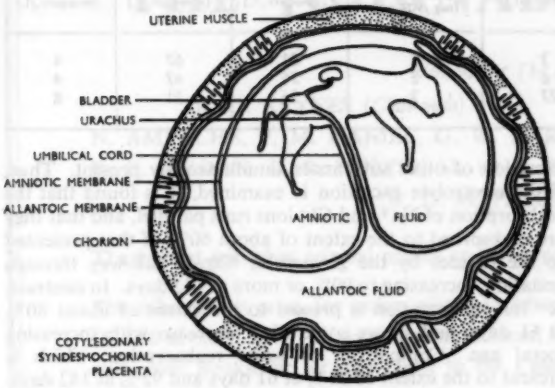
b. Relationship of the Foetus to its Amniotic and Allantoic Fluids

It is difficult to discuss renal physiology of the foetus without reference to the foetal fluids of its environment. The relationship of the sheep foetus to its foetal fluid compartments is illustrated in fig. 1.

In 1902, Jacqué compared the osmolarity of the amniotic and allantoic fluids with that of foetal urine (Jacqué, 1903–04). From these studies he postulated that in the sheep foetus the hypotonic urine would pass via the urachus into the allantoic sac up to about the 90th day of gestation (term in the sheep occurs on the 145th–148th day). Thereafter, it would pass progressively more and more into the amniotic sac, because of the increasing patency of the urethra. Alexander, Nixon, Widdas & Wohlzogen (1958a) were able to confirm this postulate.

The rate of urine production in the foetus is more than enough to account for the volume of foetal fluids observed (Alexander *et al.* 1958b). In the sheep embryo only 4 mm. in length (18 days post fertilization), mesonephric activity is present and is reflected by the presence of fluid within the

FIG. 1. Schematic Diagram Showing the Relationship of the Foetal Sheep to its Amniotic and Allantoic Fluids



allantoic sac, whereas at this time little amniotic fluid is discernible (Davies, 1952). However, the external fluid environment of the foetus must not be imagined as being due entirely to its own renal activity, as several other factors are concerned in the formation of the foetal fluids. McCance & Dickerson (1957) suggested that in the pig the allantois was secretory; indeed, Crawford & McCance (1960) recently recorded electrical potentials across the chorio-allantoic membrane of the pig, such as to indicate active electrolyte transfer. Stanier (1960) found that the composition of the mesonephric urine in rabbit and pig more closely resembled the serum than the allantoic fluid. Reynolds (1953) showed that the secretions of the buccal cavity and respiratory tract can amount to 15 ml./hr. in the foetal sheep. These and other foetal secretions may, therefore, add appreciably to the volume and composition of the amniotic fluid. Swallowing of amniotic fluid is also known to occur, and absorption along the gastrointestinal tract has been demonstrated by Nixon and Wright (unpublished). Thus, absorbed material may be incorporated into the structure of the foetus, removed from the foetal circulation by transplacental passage or voided in the urine where the cycle begins again. This dynamic interchange is illustrated by the rapid turnover of water found by Plentl (1959) in the maternal–foetal–amniotic fluid compartments in human pregnancies, where exchange with each other and in both directions is possible (see Table I).

TABLE I. Mean Transfer Rates for Water at Various Stages of Human Pregnancy (after Plentl, 1959)

(Values expressed as ml./hr.)

Relationship	12 weeks	20 weeks	30 weeks	Term
Foetus to mother	111	444	1475	3682
Mother to foetus	100	459	1619	3657
Amniotic fluid to foetus	11	78	150	149
Foetus to amniotic fluid	2	101	164	165
Amniotic fluid to mother	64	103	160	247
Mother to amniotic fluid	72	89	167	265

A living infant may be born with both kidneys congenitally absent. However, in a series of cases of renal agenesis reported recently by Bain & Scott (1960), the absence of in-utero micturition was associated almost invariably with the partial or complete absence of amniotic fluid. The inadequacy of a sufficient volume of amniotic fluid to support the foetus is demonstrated in these cases by the presence of facial characteristics (first recognized in 1952 by Potter), by talipes and by the high incidence of breech presentations.

2. Composition of the Foetal Urine

At birth the human bladder may contain up to 44 ml. (average 5.7 ml.) of dilute urine (Tausch, 1936), which is acidic and of low specific gravity. Data on the composition of foetal urine have been derived from several sources: samples obtained in cases of terminated pregnancies, first samples obtained after birth, and urine produced by the premature infant. A point worth considering in the interpretation of data obtained from bladder samples is the alteration in composition produced as the result of a variable stay within the bladder. Differences in composition between bladder samples and samples subsequently collected under experimental conditions in animals have been observed (Alexander *et al.* 1958a, 1958b; Boylan *et al.* 1958). Recently Rapoport, Nicholson & Yendt (1960) were able to show that movements of electrolytes across the bladder wall of the adult dog were passive and in keeping with the concentration gradient between the urine and the plasma. Further examination of the permeability characteristics of the bladder in the adult and foetus is clearly indicated.

Foetal urine is characteristically hypotonic with respect to the foetal plasma. This hypotonicity is accounted for in large measure by the low concentration of electrolytes, since these are reabsorbed in excess of water reabsorption. Urea and other nitrogenous end-products of metabolism are present in varying amounts. Widdowson, Dickerson & McCance (1958) obtained an average value of 80 mg. N/100 ml. of urine passed at birth, of which 72% was in the form of urea: corresponding values in the adult man were 660 mg. N/100 ml. and 85%. Proteinuria was once thought to be a fairly common feature of neonatal urine. Doxiadis, Goldfinch & Cole (1952), however, found that this was a misconception due to high urates, which gives a false positive in the method used for detecting albumin.

A systematic analysis of urine formation under controlled conditions is made possible by the use of experimental animals. Thus, in the foetal sheep, exposed by Caesarian section and with the umbilical circulation retained, a surprisingly high rate of urine flow was found. The flow in ml./min./kg. body-weight declined from 1.9 at 61 days to 0.04 at 142 days. A corresponding value for the adult sheep would be 0.02 (Alexander *et al.* 1958b). A similar decline in flow with increasing foetal age has been observed in the guinea-pig by Boylan *et al.* (1958).

The composition of bladder samples at three ranges of foetal age in the sheep is shown in Table II. We may note in these figures the increase in concentrations of urinary nitrogen and the decrease in urinary electrolytes with age.

Generalization from one species to another regarding urine composition should be avoided, since McCance & Widdowson (1957) have pointed out that in fast-growing animals the balance between anabolism and catabolism may be more

TABLE II. Average Values Obtained in Bladder Samples in Three Foetal Age-Ranges in the Sheep

Foetal age (days)	Osmotic pressure (m-osmole/l.)	Total N (mg./100 ml.)	Urea (mg./100 ml.)	Creatinine (mg./100 ml.)	Total phosphorus (mg./100 ml.)	Na^+ (m-equiv./l.)	Cl^- (m-equiv./l.)	K^+ (m-equiv./l.)
81– 93	239	76	102	3	9	81	67	4
104–117	207	110	123	6	2	71	47	4
130–142	116	180	287	27	3	26	21	8

important than the kidney in the maintenance of homoeostasis in the new-born, while in man (slow-growing) the kidney is relatively more important. In discussing renal function before birth McCance & Widdowson (1953) suggested that the composition of the foetal urine would indicate that the tubular cells were outside the control of the antidiuretic hormone, the parathyroid and possibly the supra-renals, either through immaturity or lack of circulating hormones.

3. Plasma Clearances

There has been considerable controversy over the basis upon which the immaturity of function in the neonatal kidney should be assessed with respect to the adult. The usual method for human data is to relate the values obtained to those found in an adult having a surface area of 1.73 sq. m., although it has been suggested that a better comparison might be on the basis of body-water (McCance & Widdowson, 1952). Average values for clearance of several substances have been compiled and discussed by Weil (1955). Table III, based upon Weil's data, clearly demonstrates the immaturity of the human kidney up to the age of about 24 months.

By cannulation of the urinary bladder in the sheep foetus under the conditions previously described, it has been possible to determine the plasma clearance of endogenous and exogenous substances over a period of several hours. Alexander *et al.* (1958b) found that, under these conditions, urea, fructose and in some cases creatinine all gave essentially the same plasma clearances. These authors have assumed that the process of glomerular filtration is the same in the foetus as in the adult sheep; further, that the plasma clearances of these substances may be taken as a measure of the rate of glomerular filtration by the foetal kidney. It was found that the clearance rate of these substances per kg. body-weight decreased with foetal age from a value of 2.4 ml./min./kg. body-wt. at 61 days to 0.4 ml./min./kg. body-wt. at 142 days (adult, 2.0 ml./min./kg.). By measuring the rate of glomerular filtration it is possible to examine tubular reabsorption and excretion of other substances simultaneously present. Thus, if the electrolyte excretion is examined, it is found that the reabsorption of Na^+ and Cl^- ions runs parallel, and that they are reabsorbed to the extent of about 60% of that presented to the tubules by the glomerular filtrate half-way through gestation, increasing to 90% or more at 142 days. In contrast, K^+ ion reabsorption is present to the extent of about 80% at 61 days, and shows some slight decrease with increasing foetal age. Comparison of water reabsorption, which is present to the extent of 28% at 61 days and 92% at 142 days, with electrolyte reabsorption reveals that in young foetuses the electrolyte reabsorption exceeds water reabsorption and accounts for the hypotonicity of the urine.

Little is known concerning the dynamic interplay of glomerular capillary pressure, plasma-protein osmotic pressure and intra-renal pressure upon the rate of glomerular filtration in the foetus. Measurements of blood pressure and plasma-protein nitrogen in the foetal sheep would suggest that the smaller difference between these two factors may be responsible for the lower rate of glomerular filtration in foetal life.

The tubular secretory activity for exogenous substances on the part of the foetal kidney has been demonstrated in several different species by Gersh (1937), using ferrocyanide. Teased fragments from a three-and-a-half-month human foetal kidney were shown by Cameron & Chambers (1938) to be capable of concentrating phenol red intraluminally.

The clearance of *p*-aminohippuric acid (C_{PAH}) by the foetal sheep kidney in an age-range of 89–140 days varied from 0.5 to 1.3 ml./min./kg., as compared with a range of 10.0–16.1 ml./min./kg. in adults (Alexander and Nixon, unpublished). Simultaneous measurements of the glomerular filtration rate by inulin clearance (C_{IN}) gave C_{PAH}:C_{IN} ratios of 1.2–1.5 in foetuses and 1.1–1.9 in lambs between 1 and 13 days old, as compared with the adult value of 6.4. The low clearance of *p*-aminohippuric acid by the foetal kidney may be due to a low secretory capacity on the part of the tubules or to a limitation of blood flow around the secretory elements. If

TABLE III. Average Clearance Values at Different Ages in Man (after Weil, 1955)

Age	Clearance (ml./min./1.73 sq. m.)				Tm* (mg./min./1.73 sq. m.)	
	Inulin	Urea	*p*-Amino-hippurate	Phosphate	*p*-Amino-hippurate	Glucose
Premature	45	25	150	—	13	—
New-born	40	30	200	7	25	60
3 months	70	45	300	—	40	170
12 months	—	60	550	—	70	—
24 months	—	70	650	15	75	—
Adult	125	70	650	17	75	350

* Tm: maximal capacity of tubules to secrete or reabsorb substances

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the former is correct, then the use of *p*-aminohippuric clearance to measure renal blood flow is not valid. However, measurements of renal blood flow, using an oncometer, gave values approximately in the same range as that deduced by the clearance of *p*-aminohippuric acid, which suggests that there is indeed some restriction of renal blood flow in the sheep foetus. However, Fergusson (1952) observed no essential histological difference between the intra-renal vascular pathways of adult and late foetal rabbits and guinea-pigs.

4. Diuresis and Antidiuresis

a. Diuresis

A diuresis is typically considered to be the increase in urine flow following administration of a fluid-load to the body. In adult man the glomerular filtration rate remains constant, while there is a fall in the amount of water reabsorbed, and the osmotic concentration of the urine may be lowered to a variable degree depending upon the form of diuresis—water or osmotic.

The new-born human infant, either full-term or premature, responds qualitatively in this adult manner to a water-load only when it is 6–18 days old (McCance, Naylor & Widdowson, 1954; Calcagno, Rubin & Weintraub, 1954). Infants under three days of age show no diuretic response to a water-load. However, between this age and the development of an adult response there is a gradual increase in the water excretion, but this is associated with an increase in glomerular filtration rate and little reduction in the specific gravity of the urine (Ames, 1953).

In contrast, the new-born guinea-pig, which is more developed than the human being at birth, is able to respond to a water-load in the adult manner, except that there is some increase in glomerular filtration rate associated with the increase in urine flow (Dicker & Heller, 1951).

Falk (1955) found that the rat, which is born less mature than the human being, shows no diuretic response to water in the first two days of life, but gradually develops an adult response by 10–14 days of age. However, the adult type of response could be induced in two to three-day-old rats by giving adrenal medullary hormones, which resulted in a more marked increase in urine flow than in inulin clearance. In the last two days of foetal life of the rat, Wells (1946) was able to double the rate of urine flow in two hours by subcutaneous injection of a 50% solution of urea, but analysis of the urine so produced was not performed.

Urine flow has been experimentally increased in the sheep foetus, both by direct intravenous infusion of hypotonic sulphate to the foetus and indirectly by infusion to the mother (Alexander and Nixon, unpublished). In these experiments the glomerular filtration rate increased, but not as markedly as the urine flow.

From the above data it is apparent that the stage in development at which the glomerular filtration rate becomes stabilized appears to vary from one species to another. This stage does not develop at the same time as the tubular ability to produce a urine of low osmolarity in response to a water-load. As the urine output may be increased by a reduction in tubular reabsorption or by an increase in glomerular filtration rate, the response of an animal to a water-load at any particular time will be determined by the relative maturity of these two factors.

b. Antidiuresis

Wirz (1957) explains the formation of concentrated urine on the basis of a "hairpin counter-current system" involving the loops of Henle, the vasa recta and the collecting ducts. This requires only an active sodium reabsorption and, consequent upon this, a passive water reabsorption, the latter being facilitated by the action of vasopressin in increasing the tubular and collecting-duct permeability to water. In order that this system may be effective there must be adequate structural and functional development of the tubules, and the production and release of vasopressin must be adequate. The amount of osmotically active solute present in the urine will determine the absolute osmolarity attained.

The information available on the concentrating ability of the kidney early in life should be considered in relation to the above hypothesis. The relationship between mother and foetus in utero is such that it is difficult to reduce the water available to the foetus from the mother. Urine produced by sheep foetuses near term was never found to exceed 610 m-osmole/l., and intramuscular vasopressin did not increase this value (Alexander and Nixon, unpublished).

During a diuresis induced in two foetuses of 120 and 130 days' gestation (full term, 147 days), intramuscular vasopressin produced a fall in glomerular filtration rates towards the resting levels as well as a fall in urine flow, but the older foetus showed an increase in the percentage of water reabsorbed, and the urine which had become hypotonic during the diuresis became slightly hypertonic.

Falk (1955), in her study of new-born rats, found that no response to Pitressin was shown until three days of age. This coincided with the appearance of a diuretic response to a water-load. She judged the antidiuretic response on the basis of the decrease in percentage of water-load excreted and the increase in chloride concentration of the urine.

As has already been stated, the human foetus produces a hypotonic urine before birth, while immediately after birth and during the first two days the urine is slightly hypertonic (Heller, 1944; McCance & Widdowson, 1953). Premature infants given no water or food for 2–3 days after birth showed a maximal urine concentration of 618 m-osmole/l. (Smith, Yudkin, Young, Minkowski & Cushman, 1949), compared with a serum osmolarity of 300 m-osmole/l. (Adult man has a similar serum osmolarity, while the urinary osmolarity can attain 1,400 m-osmole/l.) Ames (1953) found that withholding fluid for only eight hours in infants less than three days old resulted in the appearance in the urine of considerable antidiuretic material, although there was no reduction in urine flow. She obtained no response to exogenous antidiuretic hormone in these infants. However, after three days, when it was possible to produce some diuretic response, it was also possible to obtain a reduction in urine flow with Pitressin. But, unfortunately, the glomerular filtration rate was not recorded.

Recently, the maximal rate of reabsorption of osmotically free water during a vasopressin and mannitol infusion has been determined in infants between six and 39 days old (Edelmann, Barnett & Troupkou, 1960). The values they obtained approach adult values. Therefore, there is no difference between infants more than six days old and adults, in the effect of antidiuretic hormone on tubular permeability to water. It appears that onset of the ability to produce a diuresis and to respond to vasopressin coincide, and the vasopressin may rapidly approach adult values. However, as

antidiuretic activity has been demonstrated in the urine before a tubular sensitivity can be shown, there may be factors other than tubular maturity involved. These may include the low glomerular filtration rate and low solute excretion of earlier life.

Edelmann *et al.* (1960) also studied the effect of varying the dietary protein and water intake on renal concentration ability and have been able to produce urines with an osmolarity as high as 1,139 m-osmole/kg. with a high-protein and low-volume diet. In one premature infant they obtained similar concentrating ability by the addition of urea to the diet instead of high-protein intake. Normally, the percentage of total nitrogen excreted as urea is lower in the new-born and young of man, the pig and the dog, compared with the adult of the species (Widdowson *et al.* 1958). The concentration of urea in the urine is also greatly reduced.

Since urea represents a large fraction of the solute contributing to the high osmolarity of adult urine, this low urea excretion in the new-born accounts in large measure for the low urinary osmolarities recorded in the infant.

It is also well known that the urea clearance is lower than the inulin clearance in infancy, as in the adult, and that the ratio of urea to inulin clearance varies with urine flow and, to some extent, with the dietary protein intake. It is perhaps worth recalling that a similar condition exists in the adult sheep, where the urea clearance shows a direct relationship with the protein intake and rate of urine flow (Schmidt-Nielsen, Osaki, Murdaugh & O'Dell, 1958). These authors consider that the regulation of urea excretion in the sheep is at the tubular level and they favour the hypothesis of an active tubular transport accentuated by a counter-current multiplier system. Perhaps some similar tubular activity or permeability change, possibly regulated by protein intake, may be invoked to account for the very low rates of urea excretion found in the foetus and neonate of certain species.

5. Acid-Base Balance

The acid-base balance of the foetus is regulated ultimately by the maternal lungs and kidneys. It is known that the CO_2 tension of the foetal blood is somewhat higher than that of the maternal blood in the "steady state", and changes in the pCO_2 on one side of the placenta are quickly reflected in the pCO_2 of the other side (Dancis, Worth & Schneidau, 1957; Goodlin & Kaiser, 1957; Blechner, Meschia & Barron, 1960). Conversely, while the bicarbonate levels on the two sides of the placenta are similar in the steady state, these levels may vary independently, as has been shown following ammonium chloride infusion to the maternal sheep (Blechner *et al.* 1960) and following maternal hypoxia in acute experiments in the sheep (Britton, Huggett, Nixon and Wright, unpublished). However, the fact that urine formed in utero is acidic had been known for many years. A full analysis of the form in which acid is present in the urine during the first week of life has been presented recently by McCance & Widdowson (1960a). They show that the percentage of acid excreted as ammonium salts is high in the first two days of life, compared with that in the adult, and this is a reflection of the very low output of phosphate in the urine at this time. Also discussed is the integration of food, growth and renal function after the onset of breast-feeding and feeding with cow's milk. McCance & Widdowson (1960b) have also investigated the carbonic anhydrase activity of various tissues in the pig conceptus, which develops a markedly acid allantoic fluid. They showed considerable carbonic anhydrase activity in the foetal metanephros, among other sites.

The low phosphate excretion in the first days of life is a continuation of the situation existing in utero, where it has been shown that, while the serum inorganic phosphorus is higher in the foetus than the mother, the foetal bladder urine contains very little phosphate in the human being (McCance & Widdowson, 1960a), pig (McCance & Stanier, 1960) and sheep (Alexander *et al.* 1958a). The plasma clearance of inorganic phosphorus in sheep foetuses retaining their umbilical circulation is extremely low, sometimes zero, thus confirming that the bladder analyses reflect foetal kidney function in this respect (Alexander and Nixon, unpublished).

Thus, it appears that, apart from organic acids, the kidney is only able to excrete acid by the production of ammonia and probably hydrogen ions in the early stages of development. It is limited in its capacity to remove acid metabolic end-products, since phosphate is not available in the urine to eliminate acid until some time after birth.

6. Summary

The evidence presented in this review shows that development of function in the metanephros is spread over a period of time—some activities appearing earlier than others.

The onset of glomerular filtration precedes tubular activity, so that large volumes of urine may be passed into the amniotic or allantoic sacs from an early age. Subsequent appearance of tubular activities results first in the production of a hypotonic, and later a hypertonic, urine. The maximal osmolarity of the urine is low by adult standards because of the low solute excretion. The renal capacity to acidify the urine is limited in the early stages, in part by the very low phosphate excretion. The glomerular filtration rate appears to be somewhat labile at first and the factors leading to its stability in later life are not known. *p*-Aminohippurate: inulin clearance ratios suggest that the blood supply to the kidney is low in foetal life in comparison with that in the adult, and this may limit its functional capacity considerably.

The stage of metanephric development at which a foetus of any particular species attains an independent existence will determine its initial renal problems.

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ENZYMIC ACTIVITY DURING EARLY DEVELOPMENT

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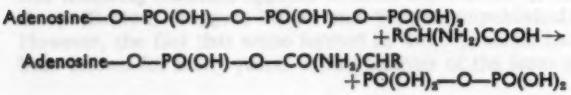
1 Synthesis of proteins and enzymes
2 Changes in enzymic activity during growth
a Mechanisms of energy supply
b Enzymes related to growth
c Enzymes related to functional metabolism
References

The distribution of enzymes in the adult varies greatly in different organs. Besides the enzymes required for the regular maintenance or renewal of the tissues, each organ is equipped with the specialized enzymic machinery required for functional activity. In the embryo the distribution of enzymes is more limited, since the primitive organs are for the most part not yet functionally mature, and metabolism is largely conditioned by the requirements for renewal or growth. The metabolism of embryonic tissue is characterized mainly by the activity of the enzymes concerned in synthesizing the proteins and lipoproteins which make up most of the organic matter of the tissue. The high activity of protein synthesis in the embryo is indicated not only by biochemical data but also by cytological evidence: the cells of embryonic tissue are commonly furnished with large active nuclei and nucleolar apparatus similar to those seen in the secretory cells of glandular tissue and in growing tumour cells.

1. Synthesis of Proteins and Enzymes

The proteins of embryonic tissue are mainly synthesized in situ from glucose and amino acids abstracted from the blood. The first step is the activation of amino-acid molecules by combination with adenosine triphosphate (ATP). The resulting amino-acyl adenylates are mixed acid anhydrides formed by a condensation reaction between the carboxyl group of the amino acid and the proximal phosphate group of the ATP. Pyrophosphate is formed as a by-product:



This reaction is catalysed by activating enzymes which are apparently specific for each individual amino acid, so that some 20 different activating enzymes are required to activate all the different amino acids required for this first step in protein synthesis. Enzymes which activate methionine, alanine, threonine, serine and other amino acids have now been prepared from different animal tissues and purified (Berg, 1956; Cole, Coote & Work, 1957; Holley & Prock, 1958). The activating enzymes are stereospecific in that they will activate only the naturally occurring L-isomers: they reject the D-isomers of the amino acids.

The subsequent steps in protein synthesis are not yet fully established, but the primary activation of the amino acids is believed to be followed by their transfer to soluble ribonucleic acid (RNA) segments which are then integrated into the organized nucleoprotein structures of the cell (Hoagland, 1960). The amino acids are thus assembled in specific genetically controlled sequences on a nucleoprotein "template", after which they are condensed by peptide linkage and peeled off continuously to form a protein molecule. The nucleoprotein structures must thus be regarded as an essential part of the enzyme system responsible for protein synthesis. Studies of the localization of protein synthesis in the cell have been made by measuring the uptake of labelled amino acids in subcellular particles separated by differential centrifugation. Amino-acid incorporation is found to be most rapid in the microsomal fraction containing a high content of ribonucleoprotein (Littlefield, Keller, Gross & Zamecnik, 1955; Clouet & Richter, 1959); but incorporation occurs also at a slower rate in the nucleus, mitochondria and soluble proteins of the cell. The localization of protein synthesis shown by this method has been confirmed in experiments by the autoradiographic method with [^{14}C]glycine and [^{35}S]methionine.

The characteristic protein particle formed in this way has been shown in a number of cases (myosin, tropomyosin, paramyosin, collagen, fibrinogen, keratin) to be a long thin macromolecule 15–20 Å in diameter and some hundreds or thousands of Ångström units in length (Schmitt, 1956). Under appropriate conditions the protein macromolecules interact with each other to form fibrous aggregates which show in the electron microscope a regular periodic cross-striated appearance characteristic for each protein. Studies of the molecular structure by *x*-ray diffraction methods have shown in some cases a helical configuration of the peptide chains; but the state of aggregation is easily affected even by small changes in the salt concentration, pH or other factors in the surrounding medium. The proteins of the cell are normally hydrated in the living state, and embryonic tissue is characterized by a relatively high water content amounting to 80% or more of the total tissue weight. But much of this water is not free: it consists of water that has acquired an ordered structure in association with protein molecules.

It used to be believed that living tissues are made up largely of fibrous "structural proteins" and that the quantities of enzymes present are relatively small; but in recent years it has become increasingly hard to draw a distinction between proteins with enzymic activity and those with a structural role. It is now known that many enzymes are intimately associated with structures such as the plasma membrane, mitochondria, endoplasmic reticulum, microsomes and even with the myelin sheath of nerve fibres (Schmitt, 1957). In many cases enzymes are inactivated if the cell structure is disturbed. Baranowski (1939) isolated from muscle tissue a crystalline protein, "myogen A", which was found on examination to possess enzymic activity as an aldolase. It was then found that the adenosine triphosphatase activity of muscle is closely associated with the myosin of the myofibrils. Since that time, many other enzymes of muscle have been isolated in the form of crystalline proteins (lactic dehydrogenase, glyceraldehyde phosphate dehydrogenase, glycerophosphate dehydrogenase, pyruvate phosphokinase) and together they account for a

large part of the total protein content of the tissue (Beisenherz, Boltze, Bücher, Czok, Garbade, Meyer-Arendt & Pfleiderer, 1953). The number of enzymes hitherto identified in the tissues adds up to a considerable figure, but it is likely that this still represents only a small fraction of the total number that are present. There is, therefore, reason to believe that in muscle, and probably in other tissues including the embryo, enzymes may make up the main bulk of the proteins in the living cell.

The nature of an enzyme is determined partly by the prosthetic group, which is generally constant for enzymes catalysing any particular reaction, and partly by the protein residue, which may affect the solubility, pK and other properties including the Michaelis constant, or its affinity towards different substrates and inhibitors. The protein residue varies with the animal species, depending on the genetically determined amino-acid sequence. A term such as "lactic dehydrogenase" thus does not represent a single molecular entity: it describes a group of enzymes that may vary not only in different species, but also in different individuals and even in different tissues of the same individual. Kalow (1959) has shown that human sera contain one or other of two types of pseudocholinesterase which can be distinguished by differences in their affinities for substrates and inhibitors. Studies of the distribution of these enzymes in different members of a family indicate that the type of esterase is genetically determined; there appear to be two autosomal allelic genes, and heterozygous subjects have a mixture of the two enzymes. Differences in the enzymic make-up of different individuals are indicated again by the genetic variation in the serum haptoglobins and transferrins (Smithies & Connell, 1959). Recent studies of the distribution of lactic dehydrogenase and other enzymes in the serum proteins separated by electrophoresis suggest that different molecular modifications of these enzymes may also occur (Wieme, 1959). Apart from differences in the amino-acid sequence, enzymes differ also in the state of aggregation of the protein molecules. The view that enzymes may function as polymers is supported by the work of Frieden (1959), who has shown that glutamic dehydrogenase is active in the dimeric state, while the individual monomers are inactive. Brenner (1959) has suggested that phosphorylase and adenylsuccinase may act as tetramers, and that the binding affinity of the monomers may be subject to genetic variation. If enzymes are active in the polymerized state there seems no reason why they should not also serve as structural units in the living cell.

2. Changes in Enzymic Activity during Growth

a. Mechanisms of Energy Supply

Energy is needed by living cells, in the first place for the synthesis of proteins and other metabolites during growth, and later for functional activity; it is needed also at all stages of development for purposes of tissue repair, and for the performance of osmotic work in maintaining the normal ionic balance between cell and interstitial fluid. The range of substrates utilized by the embryo as a source of energy is at first very limited. Needham (1942) found that, besides glucose, mannose was the only substrate utilized by the four-day-old chick embryo to any substantial degree; glycogen and other carbohydrates were completely unattacked. As development proceeds, there is evidence of a shift of energy sources from carbohydrate to protein and from protein to fat. This is indicated by the fall from 1.0 to about 0.7 in the respiratory quotient (ratio of CO_2 formed to O_2 uptake) and also by the amounts of different substrates utilized: thus it can be shown that during the three weeks of incubation more than 90% of the total material oxidized by the chick embryo consists of fat.

The energy derived from the metabolism of carbohydrate, protein or fat, is used to synthesize the "high energy" bonds of ATP, which is the form in which energy is made available for most of the different kinds of work performed by the cell. The six-day embryo has been shown to contain inorganic phosphate, ATP, phosphocreatine, hexosediphosphate, pyruvate and most of the enzymes required in the Embden-Meyerhof scheme for the glycolysis of glucose (Needham & Lehmann, 1937; Meyerhof & Perdigon, 1940). Glycolytic activity is high in the chick embryo of 4–6 days and it would appear that almost all the energy required for the synthesis of ATP is at first obtained by the utilization of glucose by glycolysis; in agreement with this, the pH of the chick's amniotic and allantoic fluids rapidly falls and the lactic acid content is found to rise to as much as 80 mg./100 g. (Needham, 1942).

Mitochondria are present in the embryo from the time of fertilization of the ovum, but they are at first relatively few in number and little or no cytochrome oxidase or cytochrome *c* is found in the embryo for the first 5–6 days. At about the sixth day mitochondria begin to appear in increasing numbers and the oxidation of glucose overtakes glycolysis; this is a more efficient process, since it yields about ten times the amount of energy per molecule of glucose. The six-day embryo shows the Pasteur effect—the inhibition of glycolysis under aerobic conditions—and hence the ability to adjust to anoxia by switching from oxidation to glycolysis. The increase in mitochondria is associated with the appearance of the enzymes of the citric acid cycle and the electron transport chain: these include succinic dehydrogenase, fumarase, aconitase, oxaloacetic decarboxylase and the transaminases, as well as flavoprotein and cytochrome oxidase. Mitochondria have been regarded as systems primarily concerned with the generation of ATP by oxidative phosphorylation. They vary to some extent in their properties in different tissues, but generally they are able to utilize a large variety of amino acids and fatty acids as well as carbohydrates and other intermediate metabolites.

During foetal life there is a gradual increase in the activity of the oxidative mechanisms, but the extent of the increase varies in different tissues, depending on the time at which they mature and on their ultimate energy requirement for functional activity. Maximal cytochrome oxidase activity is attained in the brain of most mammalian species at a few weeks after birth, but the increase is not regular over the whole period of development. Flexner (1955), who measured the activity of the cytochrome oxidase and other mitochondrial enzymes in the brain of the guinea-pig during foetal development, found a sudden increase in the activity of these enzymes at a certain "critical period" associated with the maturation of the cortical neurones. Dawkins (1959) has shown that there is a similar sudden increase in the number of mitochondria, and in the activity of the mitochondrial enzymes, in the rat liver during the neonatal period. He found a threefold to fourfold increase in the activity of the respiratory enzymes during the first few days of life. This may be related to the increase in functional activity of the liver after birth.

b. Enzymes Related to Growth

There are a number of enzyme systems that are active in the embryo, but which disappear with the cessation of growth, so that they are absent or only barely detectable in the adult. Srere, Chaikoff, Treitman & Burstein (1950) found that there was an active synthesis of cholesterol in the growing tissues of the new-born rat, but in adult tissues the synthesis was negligible. Similar findings have been reported for certain enzymes concerned in amino-acid metabolism. The brain of the new-born mouse shows an active incorporation of ^{14}C from labelled glucose into cystine, histidine, lysine and leucine; but this activity is absent in adult tissues (Winzler, Moldave, Rafelson & Pearson, 1952).

The mechanism of protein synthesis has already been discussed. Measurements of the rate of uptake of labelled amino acids by the tissue proteins suggest that the protein-synthesizing systems are much more active in the embryo than in the adult. Zamecnik, Frantz, Loftfield & Stephenson (1948) showed that foetal liver slices incorporate [^{14}C]alanine and [^{14}C]glycine at a high rate, approaching that of a hepatoma; the rate is many times higher than in the adult liver. Greenberg, Friedberg, Schulman & Winnick (1948) found that, in the 13-day chick embryo, incorporation of [^{14}C]glycine is about twice as fast in the brain as in the liver (see Table I). This agrees with the observation that the growth of the brain proceeds ahead of the other organs of the body: in the five-day embryo the brain accounts for as much as 30% of the total body-weight. In the adult, amino-acid incorporation continues to be active in organs such as the liver, which are concerned in protein formation, but it falls to a relatively low level in muscle, cartilage and other tissues.

A high rate of amino-acid incorporation suggests that the protein-synthesizing systems are active; but protein degradation is proceeding at the same time, and growth depends on the difference between the rates of these two processes. Amino-acid incorporation is not entirely satisfactory as an index of protein synthesis, since it is possible that amino acids may enter a protein molecule by an exchange reaction which does not involve the complete synthesis of the whole protein molecule. Development has also been shown to involve phases of morphogenetic degeneration in which large numbers of the cells degenerate and die. Numerous cell deaths are seen, for example, during the process of formation of the neural groove (Boyd, 1955), and similar phases of degeneration are found in the normal course of development up to a much later stage. Uzman & Rumley (1958) showed that the growth of the mouse brain is interrupted by a phase between the 16th and 23rd day of life, during which there is a considerable decrease in the number of cells, with a corresponding fall in deoxyribonucleic acid (DNA); at this stage there is also a decrease in water content and a decrease in the fresh weight of the brain. The changes in cell population occurring in this way are bound to cause changes in the distribution of enzymes, and this may account for some of the minor changes in enzymic activity during growth. Enzymic activities are sometimes expressed in terms of the concentration (enzyme units/ml.) or in terms of the amount of substrate reacting per minute per unit of fresh weight; but clearly it is unsatisfactory to express activities in this way where there are considerable changes in water content, such as occur during growth. The expression of enzymic activities in terms of the dry weight or of the protein of the tissue can also be misleading if changes in cell population are not taken into account. In any case it must be remembered that tissues are not homogeneous media. The distribution of an enzyme in an organ is often irregular and its activity in vivo will be affected by its relation to the various compartments of the cell.

TABLE I. Incorporation of ^{14}C from [^{14}C]Glycine into the Proteins of Homogenates of Chick Tissues

	Counts/mg. protein/min.		
	5-Day embryo	13-Day embryo	Newly-hatched chick
Liver	180	50	31
Brain	180	104	38

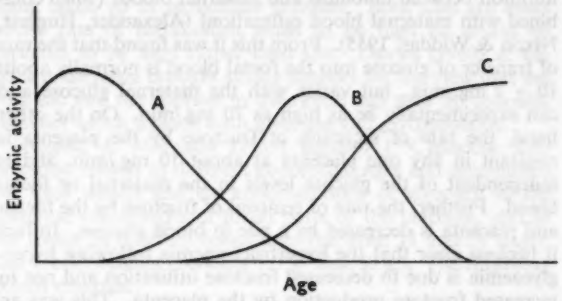
Modified from Greenberg et al. (1948)

c. Enzymes Related to Functional Metabolism

Some enzymes that are absent or present in low activity in the embryo increase greatly in activity at the time when an organ becomes functionally mature, and then remain active throughout life; it is reasonable to associate such enzymes with functional activity rather than with processes of growth. Ashby & Schuster (1950) found that carbonic anhydrase increases enormously in activity in the rat brain during the first few weeks of life, while it is absent in the foetus and at birth. Similar findings have been reported for the amine oxidase, which is present in the mitochondrial fraction and which increases in activity in parallel with succinic dehydrogenase and other mitochondrial enzymes (Nachmias, 1960). Acetylcholinesterase, which is associated with functional activity of the nervous system, increases in activity for several weeks after birth in the brains of the rat and rabbit (Himwich & Aprison, 1955), but it appears in nervous tissues at certain other sites before it is active in the brain. Bonichon (1958) reported the presence of acetylcholinesterase as early as the fifth day of life in neuroblasts of the spinal cord of the chick embryo, and Nachmansohn (1940) found it in the spinal cord of the sheep foetus of 60 days at a much higher activity than in the brain. This relationship changes as development proceeds. The cholinesterase activity of the cord diminishes while that of the brain increases until the activity in the brain, and particularly in the caudate nucleus, far outstrips that of the cord. The changes may be related to the fact that the lower centres in the spinal cord reach functional maturity before the higher centres of the brain.

A late-developing enzyme which is of some clinical interest is glucuronyl transferase. This enzyme effects the detoxication of a number of toxic compounds including bilirubin: it is present in the microsomal fraction of the liver. Brown & Zuelzer (1958) showed that glucuronyl transferase is absent in foetal liver and present only in traces in the new-born, but that it increases rapidly in activity in the neonatal period. As with other enzymes, the time of development of the enzyme is controlled by genetic factors. If severe jaundice occurs in the neonatal period before the activity of the enzyme is sufficient to conjugate the bilirubin in the blood, kernicterus may result and the infant is permanently damaged. The source of the glucuronic acid transferred by the enzyme is uridine-diphos-

FIG. 1. Changes in Enzymic Activity During Development



Curves of type A are obtained for enzymes concerned mainly with growth.

Curves of type B are given by enzymes concerned in functional activity.

Curves of type C are obtained for enzymes concerned with processes of maturation.

phate-glucuronic acid (UDPGA), which is formed by the action of another enzyme UDPG-dehydrogenase; this enzyme is also deficient in the foetus and it increases in activity in the period after birth.

The changes in activity of different types of enzymes during development are indicated diagrammatically in fig. 1. Enzymes primarily concerned with growth are most active in the embryo and they give activity curves of type A: enzymes concerned with function develop later and give curves of type B. There are also a number of enzymes which show a sharp peak of activity at an intermediate stage before the tissues are functionally mature: it is likely that these enzymes may be concerned in effecting the morphological and other changes associated with processes of maturation. A function of this kind has been ascribed to the glutamotransferase (Rudnick & Waelsch, 1955) and the alkaline phosphatase of the brain (Cohn & Richter, 1956). Besides the changes in enzymic activity, maturation involves changes in the organization of enzymes within the cell. Thus at about the tenth day after birth, the glutamic dehydrogenase and other enzymes of the soluble cytoplasmic fraction of the rat liver become more closely attached to the mitochondria (Solomon, 1960).

As development proceeds and the different organs of the body become functionally mature, the metabolism of growth which predominates in the embryo is gradually replaced by functional metabolism of a more highly specialized kind. The change from one type of metabolism to another is achieved by the successive appearance of new enzymes, which are released as differentiation proceeds and the corresponding genes mature. The changes in metabolism may be considered in terms of ascending and descending curves of enzymic activity, which effect a gradual change from one metabolic pattern to another.

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CARBOHYDRATE METABOLISM IN THE PLACENTA AND FOETUS

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1 Formation of fructose
2 Placental glycogen
3 Carbohydrate enzymology of the placenta
4 Role of galactose
5 Comparative distribution of fructose
6 Fate and function of fructose and glycogen
References

This paper confines itself to a limited aspect of carbohydrate metabolism in the foetus. It deals with the fact that the bloods of the foetus in different species have one or two sugars, glucose (dextrose, an aldohexose) and fructose (laevulose, a ketohexose), and that in the placenta there is a store of glycogen. These carbohydrates appear to have a special function and to be available only to the foetus, not to the mother. Their comparative distribution is of interest and is in consequence considered. The paper does not deal with the mechanism of transfer of carbohydrates across the placenta, still less with the energetics of carbohydrate metabolism in the foetus.

The year 1946 marked a change of phase in our knowledge of the physiology of carbohydrate metabolism of the foetus, for in that year Bacon & Bell published their unequivocal chemical identification of the sugars of the blood of the sheep foetus as glucose and fructose (see also Bacon & Bell, 1948). This stimulated a number of lines of work, and the history of this work up to 1951 is summarized by Huggett (1955).

1. Formation of Fructose

Fructose disappears from the blood of the sheep within 24 to 48 hours of birth (that is, falls to 1 or 2 mg./100 ml.) (Cole & Hitchcock, 1946). It was shown to be secreted by the placenta in vivo into the umbilical circulation—when glucose was given intravenously to the mother or the foetus (Huggett, Warren & Winterton, 1949). Further, the fructose enters the foetal circulation from the placenta, even when the placenta is perfused with a pump through the umbilical artery, the pump replacing the foetus (Huggett *et al.* 1949). Alexander, Huggett & Widdas (1951), using [^{14}C]glucose, showed that the placenta formed labelled fructose and, if no carrier was used, did so without hyperglycaemia, at normal levels. This was confirmed by Alexander, Andrews, Huggett, Nixon & Widdas (1955). If the placenta is perfused there are two systems which can be used. The perfusion fluid can leave the reservoir and pass through the placenta and back by the umbilical vein to the smaller reservoir ("through-perfusion") or return to the same reservoir ("re-circulating-perfusion"). The former gives information as to rates of secretion or removal of substances by the placenta, the latter as to equilibration between umbilical and maternal bloods (when combined with maternal blood estimation) (Alexander, Huggett, Nixon & Widdas, 1955). From this it was found that the rate of transfer of glucose into the foetal blood is normally about 10 ± 2 mg./min., but varies with the maternal glucose and can experimentally be as high as 70 mg./min. On the other hand, the rate of secretion of fructose by the placenta is constant in any one placenta at about 10 mg./min. and is independent of the glucose levels in the maternal or foetal blood. Further, the rate of removal of fructose by the foetus and placenta is decreased by a rise in blood glucose. In fact it became clear that the hyperfructosaemia following hyperglycaemia is due to decreased fructose utilization and not to increased fructose production by the placenta. This was an interesting, unexpected, finding. Incidentally, it was noted that normal and experimental in-vivo umbilical fructose levels correlated better with the umbilical glucose than with the maternal blood glucose.

In the rhesus monkey and the human subject, on the other hand, the umbilical blood fructose was always less than 10 mg./100 ml., and usually under 5 mg./100 ml. Further, it always kept low despite experimental elevation of the blood glucose in the umbilical blood following intravenous injection into the mother (Chinard, Danesino, Hartmann, Huggett, Paul & Reynolds, 1956). This response is the cardinal difference between the fructogenic or macro-fructose types, such as the sheep, and the other types, which do not produce hyperfructosaemia, but have a high placental glycogen (glycogenic).

Since hyperglycaemia does not increase the rate of secretion of fructose into the blood, and blood levels are the resultant of inflow and removal, then it follows that the rise of blood fructose following hyperglycaemia must be due to decreased rate of removal. In other words, fructose utilization is replaced by glucose utilization if extra glucose is supplied. This fits in with the findings of Jonas (unpublished) and of Long (1957), in which foetal red cells in vitro use glucose in preference to fructose, and only use the fructose when the glucose content has fallen very considerably. Karvonen & Leppänen (1960) have since shown that galactose, too, inhibits fructose utilization.

2. Placental Glycogen

Originally described by Bernard (1859) in the rabbit, the relative constancy of placental glycogen in comparison with maternal hepatic glycogen was shown by Lochhead & Cramer (1908) and by Huggett (1929) and Davey & Huggett (1934). Szendi (1934) thought the placental glycogen correlated with a high rate of metabolism, whereas Dempsey & Wislocki (1945) decided it was correlated with relatively low rates of metabolism, an exactly opposite point of view. Villee (1953a, 1953b) has studied its deposition in the human placenta taken as a whole, and has shown that it declines from about the 10th–12th week of pregnancy towards full term. With this there is also a decline of the glucose-6-phosphatase and also of the rate of oxygen utilization by the placenta, which tends to support Szendi's interpretation rather than that of Dempsey and Wislocki.

Hertig (1960) and Boyd (1960) independently, at a conference in New York in 1959, described the presence of glycogen in early human embryos, Hertig in the germinal disk in

a 13-day embryo, and Boyd in the gland cells of the uterine epithelium and in the tips of the villi at about the third month. Both writers were in agreement on the possibility of glycogen's being taken up as particulate granules. Both sites are areas whose rate of metabolism tends to be high or would be expected to be high rather than low. Davies (1960) at the same conference brought strong support for the view that the yolk sac and the chorion in the rabbit can take up colloidal particles which include both glycogen and glycoproteins.

Huggett & Morrison (1955) injected [^{14}C]glucose into pregnant rabbits at different conceptual ages and determined the total weight and the concentrations of glycogen in the maternal (decidual) and foetal parts of the placenta, and in the foetus at different foetal ages (between 18 and 28 days), and foetal weights (between 1.5 g. and 30 g.). The following points emerged:

i. Radioactive glucose given to the mother goes normally to the maternal placenta up to the 18th day.

ii. Between the 18th and 22nd day it goes also to the foetal placenta and foetus, decreasingly to the former and increasingly to the latter. On the whole, the foetal-placental counts exceed the foetal, although in this period there is considerable irregularity and apparent activity.

iii. From the 25th day onwards little or no glycogen is accumulated or synthesized anywhere, least of all in the maternal placenta.

iv. The placental glycogen is in a state of continuous exchange with the maternal blood glucose.

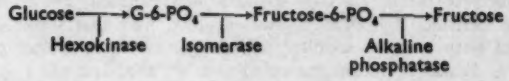
v. The total glycogen formed in tissue examined between the moment of injection and the chemical analysis of the mother and foetus ($\frac{1}{2}$ to 4 hours) is maximal in the maternal placenta at 5 g. foetal weight (21 days) and in the foetal placenta at 1.5 g. foetal weight (18 days); there is a second maximum at 6 g. foetal weight (21 days, 12 hours).

vi. The pattern of glycogen deposition in the two parts of the placenta is quite different (the decidual preceding the syncytial with different specific activities). This indicates that the two depositions are independent formations, and confirms a suggestion of Lochhead & Cramer (1908). The total rates of glycogen formation vary in different stages of development and do not vary in parallel in the two parts of the placenta.

Buck (1959) utilized the glucose oxidase method of determining glucose in order to estimate the glucose in copper-reducing glycogen hydrolysates from placental and foetal membranes of different animals, coupling it with chromatography to assess the other products of hydrolysis. It would appear that the tissues could be divided into glycogen-rich and glycogen-poor groups. The former, on hydrolysis, yielded glucose only. The glycogen-poor group yielded other reducing sugars. In the former class was the glycogen of the rabbit placenta. In the latter were the glycogens from sheep placenta and the foetal membranes of sheep and rabbits; the non-glucose reducing sugars could exceed the pure glucose by as much as 4:1. These include galactose, mannose, fucose, xylose, inositol and sorbitol, all identified chromatographically. All could come from the cell membranes; however, the first three may come from mucopolysaccharides, and the galactose additionally from galactogen.

3. Carbohydrate Enzymology of the Placenta

The first obvious line of attack is the applicability of the Embden-Meyerhof-Cori cycle to the placenta.



Hexokinase

There has been failure to show adequate hexokinase in placental extracts of any species unless adenosine triphosphate is added (Wajzer & Zelnik, 1951); this appears to be normally inadequate in both sheep and human placentas.

Isomerase

This has not been demonstrated in sheep placenta, but Wajzer & Zelnik (1951) have shown it to be present in human placental extracts; the human placenta fails to form fructose in the way that the sheep placenta does. In human placental extracts these workers obtained conversion of 30% of glucose-6-phosphate to fructose-6-phosphate.

Alkaline phosphatase

Ainsworth, Parr & Warren (1951) found this enzyme widely distributed in all types of placenta, but ironically in maximal concentration in those species producing no fructose, and in minimal concentration in the fructogenic species. Fahmy (1953) found it histologically in the sheep's maternal (decidual) placenta and absent in the chorionic villi and other foetal parts. But in the human placenta, on the other hand, alkaline phosphatase is absent in the maternal parts but present in the syncytiotrophoblast, accompanied by many mitochondria. It is never present in the same cells as glycogen (Fahmy & Huggett, 1954).

Further, Villee (1953a) has found that the human placenta in early pregnancy has both high glycogen and high glucose-6-phosphatase, and both decline from the 10th week to full term, together with a decline of metabolic rate. Similarly, Hagerman & Villee (1952) demonstrated that human placental extracts in vitro form fructose (whereas Chinard *et al.* in 1956 demonstrated this does not happen in vivo in the two primates—rhesus monkey and man).

Summing it up therefore, the Embden-Meyerhof-Cori cycle does not appear to explain how the fructogenic species form fructose in vivo. It does, however, begin to explain the formation of glycogen in the glycogenic species. There is no evidence either way on the presence of phosphoglucomutase in the placenta, which would account for the glycogen synthesis. Much more work needs to be done on the degree to which this system is applicable to the two types of placenta.

The sorbitol pathway

Hers (1957a, 1957b) suggested that the sheep's placenta converted glucose to sorbitol with an aldose reductase and that sheep's foetal blood had 150 mg./100 ml. of sorbitol. The foetal blood went to the liver, where the sorbitol was converted to fructose with a sorbitol dehydrogenase present there. Hers showed that the aldose reductase, but not the dehydrogenase, was present in the sheep's placenta. Thus the fructose formation is a two-stage process in two different organs.

The finding that sheep's placenta could form sorbitol was

confirmed in vivo by Andrews, Britton & Nixon (1959), who demonstrated that perfusion of the placenta by re-circulation methods through the umbilical artery, while experimental hyperglycaemia in the mother was produced, yielded sorbitol. Further sorbitol was isolated, cystallized and identified by *x*-ray crystallography, and also shown to react with purified sorbitol dehydrogenase from guinea-pig liver. It was therefore identified as D-sorbitol and not L-sorbitol. The normal concentration, however, was only of the order of 10 mg./100 ml. and not therefore at the high level of 150 mg./100 ml. reported by Hers. Andrews, Britton, Huggett & Nixon (1960) have carried this further and shown that the sheep's foetal liver perfused with sorbitol forms fructose, but if perfused with glucose it does not form fructose, nor does it take up fructose if this sugar is perfused through the foetal liver. Galactose and inositol did not form fructose when so perfused.

The ability to form fructose by this method of synthesis does not, however, explain the ability of the placenta in the absence of a foetal liver to form fructose, when perfused with glucose through the umbilical vessels.

Hagerman, Roux & Villee (1959) investigated by in-vitro methods the possibility that Hers' sorbitol pathway might apply in the human placenta at full term. They found absence of aldose reductase but presence of ketose reductase, and therefore the first stage of the sorbitol pathway could not operate. There is also no significant amount of sorbitol in the human placenta. On the other hand, while the homogenates of the human placenta can convert fructose-6-phosphate to phosphate and fructose, they can also convert glucose-6-phosphate to glucose and phosphate; but they do not contain a specific fructose-6-phosphatase. It would seem as if, so far as the human placenta is concerned, fructose could be formed from fructose-6-phosphate with the aid of a non-specific phosphatase, but in practice no considerable quantities (> 10 mg./100 ml.) are found. Nor does hyperfructosaemia follow hyperglycaemia (Chinard *et al.* 1956).

4. Role of Galactose

Beyond the fact that some galactose can be got from foetal membranes after hydrolysis with potash, precipitation with alcohol and hydrolysis of the polysaccharides, followed by chromatography, there is no evidence to date that galactose or uridine phosphates play a part (not that galactose is a likely route to fructose) as sources of glucose or fructose. There is nothing to exclude this possibility, but the fact that Buck (1959) found no galactose in glycogen-rich tissue, and only in glycogen-poor membranes (such as sheep cotyledons), suggests that galactose may be a component of the cell membrane—possibly a mucopolysaccharide but not derived from glycogen.

5. Comparative Distribution of Fructose

Ainsworth *et al.* (1951) first showed that sheep and goats were fructogenic, whereas rats, guinea-pigs and rabbits were not. Walker (1954) extended this to three species of deer, roe, fallow and Père David, where fructose in the foetal fluids ranged from 100–300 mg./100 ml. Goodwin (1956) demonstrated that the baleen whale, the horse and pig, as well as the ox, goat and sheep, were fructogenic, while the cat, dog and ferret, rat and rabbit were relatively free of fructose. Huggett (1959a) extended his studies on fructose to the odontocete whales, to the hippopotamus and to the antelope (kob), together with the fin and sei whales of the Mysticeti. Since then Huggett & Nixon (1961) have added to this list another hippopotamus, the rhinoceros, the camel and the llama. But in the Edentata, the sloth (*Bradypus tridactylus*) only had traces of fructose in the foetal blood.

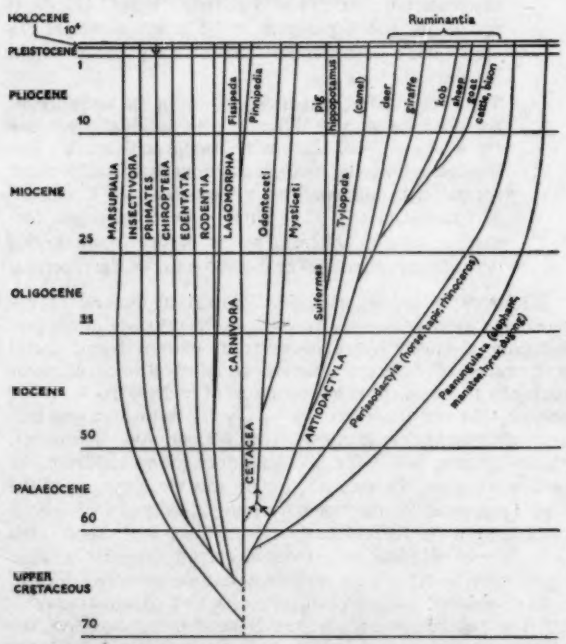
The yardstick of whether a species is fructogenic or not is not that chemical traces of fructose are present or absent, but that it is found in quantity in the foetal blood (> 10 mg./100 ml.) and in even greater quantity in the amniotic and allantoic fluids. Further, and most important, intravenous glucose is converted to fructose. This test is not always practicable, of course. But it has been shown that monkeys and human beings (Chinard *et al.* 1956) are not fructogenic, despite the presence of fructose in quantities of less than 10 mg./100 ml.—often less than 5 mg./100 ml.—in the foetal blood (Karvonen, 1949; Hagerman & Villee, 1952), and an in-vitro production of fructose by the placenta (Villee, 1953a, 1953b).

It is of interest to look at the fructogenic mammals in terms of their distribution among the mammalia and the present-day beliefs about the palaeontological origin of the different cohorts. (See fig. 1.)

The fructogenic groups that are known include the Cetacea, both Odontoceti and Mysticeti, and the "Ungulata", both the Artiodactyla and the Perissodactyla. So far, no opportunity has presented itself for the examination of any Paenungulata, such as the foetus of the elephant or hyrax.

It is interesting that both divisions of Cetacea have fructose; this suggests an affinity with the Ruminantia rather than with

FIG. 1. Palaeontological Tree of Development of the Mammalia, indicating the Time Relations and Origins of the Main Cohorts



Numbers on left show time expressed in millions of years, except for Holocene period (ten thousand years).

the Carnivora, namely, that in the Palaeocene the primitive Cetacea split off from the root stock, giving rise to hoofed animals. The arrowed sections in fig. 1 indicate the possible roots of origin of the Cetacea.

It would be of interest to know whether the Paenungulata which split off in the Eocene are fructogenic or not: whether in fact they derive from roots common to Carnivora or to Ungulata. If the latter, it would suggest that the fructogenic function of the placenta is a primitive mode of providing a carbohydrate for foetal growth, unavailable to the mother, which is later replaced by the water-insoluble glycogen. Quite clearly this is largely speculation at this stage, but the mosaic does indicate a pattern. There is no evidence regarding Monotremata or Marsupialia. The chick is fructogenic.

6. Fate and Function of Fructose and Glycogen

Fructose in estimations of foetal blood shows a statistical difference in the contents of the umbilical artery and umbilical vein of about 1 mg./100 ml. in sheep.

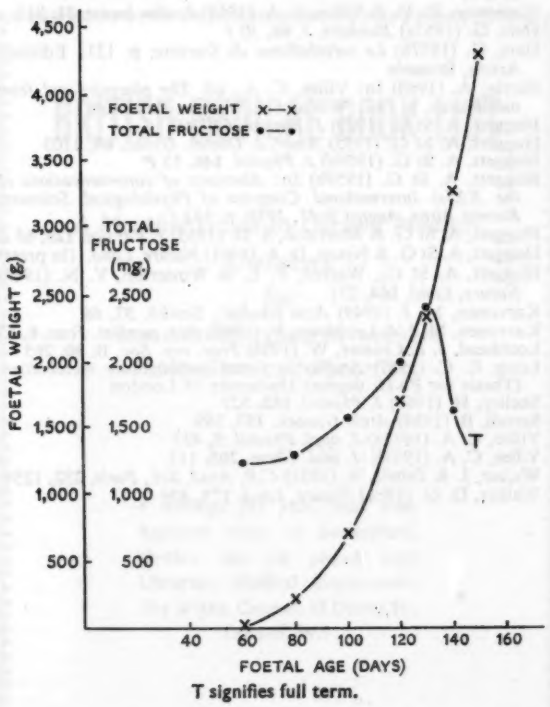
The utilization of fructose is not increased in hypoxia. Fructose is present in foetal urine and in the amniotic and allantoic fluids. It is not utilized by the foetal liver (Andrews *et al.* 1960) until the 152nd day from conception. This is unaffected by whether the birth is premature or not. The evidence for this comes from a single experiment in which a premature Caesarian delivery was performed on the 140th day, and the premature lamb was kept alive by special nursing. On the seventh day after delivery the liver was perfused and no fructose was utilized. The only factor which seems to be able to increase the consumption of fructose is deprivation of glucose, whether in foetal red cells (Jonas, unpublished) or in perfused placenta (Alexander, Huggett, Nixon & Widdas, 1955). The reverse is true: increase of glucose decreases the consumption of fructose.

Long (1957) has shown that the human and the sheep placenta handle fructose differently: human placental slices utilize fructose and glucose anaerobically, the sheep more effectively (the ratio of fructose : glucose utilization for man is 1:1, for sheep, 0.3:1). Aerobically, the sheep placenta can utilize both.

The loss of fructose by the placenta of the sheep has been shown to be negligible by Britton, Huggett and Nixon (unpublished) in several ways. Injected into the foetal blood it caused no maternal hyperfructosaemia; [^{14}C]fructose injected likewise produced no rise in maternal counts. In one unpublished experiment (Alexander, Huggett and Nixon), perfusion of the uterus via the uterine circulation when foetal hyperfructosaemia was produced gave no fructose in the pump perfusion circulation. This result excludes the maternal liver as a utilizer of fructose, which would camouflage the arrival of fructose in the maternal blood.

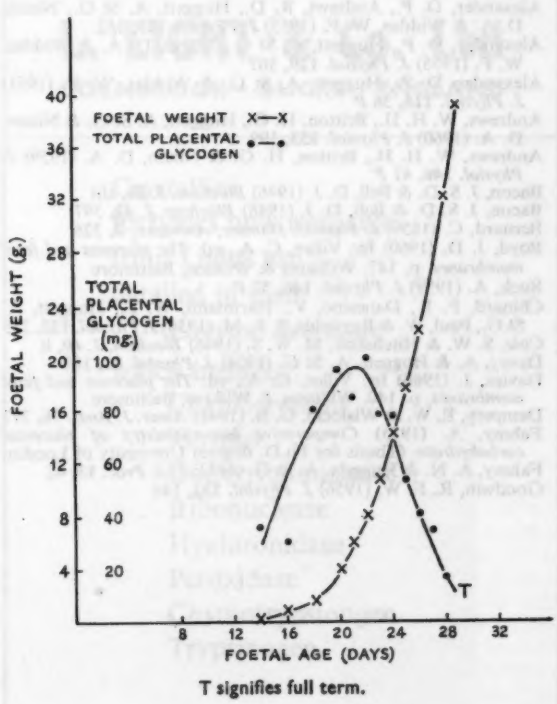
We needs must ignore mucopolysaccharides and carbohydrates, other than glycogen and fructose, such as those demonstrated by Buck (1959).

FIG. 2. Foetal Weight and Total Fructose Content of the Fructose Space at Different Foetal Ages in the Sheep



T signifies full term.

FIG. 3. Foetal Weight and Total Placental Glycogen Content at Different Foetal Ages in the Rabbit



T signifies full term.

TABLE I. Comparison of Physiological Properties of Foetal Blood Fructose and Placental Glycogen

Fructose	Glycogen
Never present in quantity with glycogen in quantity	Never present in quantity with fructose in quantity
Found in "Ungulata" and Cetacea (see fig. 1)	Found in rats, rabbits (see fig. 3) and primates
Not available to the mother	Not available to the mother
Not normally oxidized	Apparently does not fluctuate with changes in O_2 utilization
Not oxidized in hypoxia	—
Maximal concentration in sheep at 130th day	Maximal concentration in rabbit at 21st day (see fig. 3) and in man at 10th week
Disappearance in sheep synchronizes with the rapid foetal growth of the last 14–21 days	Disappearance in rabbit synchronizes with the period of rapid foetal growth in the last 8–9 days. In man this begins before the rapid period of growth
The absorption of fructose from the foetal gut and excretion in the urine is such that the "fructose space" includes the foetal fluids	—

It is now possible to compare glycogen and fructose. Table I shows such a comparison, and figs. 2 and 3 show the total fructose of the sheep foetal fructose space and the total glycogen of the rabbit's placenta, both compared with the rates of foetal growth (Huggett, 1959b).

The fact that these two carbohydrates do not appear to be oxidized, that they are unavailable to the mother, utilized by the foetus at a constant and apparently almost unvarying rate—possibly replaced by extra, free glucose if available—and reach high peaks which are followed by depression at the height of foetal growth-rate, strongly suggests that they are needed as a reserve of carbon, hydrogen and oxygen for synthesis of foetal tissue in the period of rapid foetal growth in the last days in utero. On the other hand, the ratio of the carbohydrate being used is only one-thousandth that of the tissue deposited as foetus. The carbohydrates therefore appear to be needed as reserves for the synthesis of some particular component only required in micro-quantities.

It would seem that probably all mammals partake of both these carbohydrates in their foetal membrane and foetal fluids. But with the Ungulata and whales the glycogen is minimal, and does not appear to increase in hyperglycaemia, whereas fructose does. With the rabbits, rats and primates the fructose is in mere trace quantities, and labelled glucose produces labelled glycogen. The quantities of fructose produced in the two groups of mammals are of different orders of magnitude.

Shelley (1960) has recently put forward the view that foetal muscle glycogen is a source not of aerobic energy but of carbon, hydrogen and oxygen for the synthesis of the tissue protein. This in no way conflicts with the view expressed in this paper.

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THE INFERIOR AORTICO-PULMONARY GLOMUS

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Department of Anatomy
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b The cells of the inferior aortico-pulmonary glomus
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The work of a large number of investigators in the past thirty-five years has established beyond reasonable doubt the importance of the carotid body and homologous structures in the reflex control of respiration. Recent monographs dealing with the morphology of the carotid body (Adams, 1958) and with the physiology of the peripheral chemoreceptors (Heymans & Neil, 1958) give excellent surveys of the relevant literature. There still remains, however, much uncertainty on a number of important aspects of these small organs. Thus, the basic mechanism rendering these structures sensitive to changes in the blood circulating through them still eludes us. Furthermore, there are problems of considerable interest posed by the histology of the constituent cells of these organs; their precise origin is not fully established and their distribution requires analysis. Finally, very little is known about what function, if any, is subserved by them in mammals during foetal life and at the time of birth. The present contribution deals with several of these problems in relation to one of the intra-thoracic structures which, in man, is generally considered to be homologous and analogous to the carotid body.

1. Terminology

There are two separate terminological problems posed by the carotid body and its homologues. The first difficulty relates to the actual nature of the tissue, or tissues, contained within these organs. Earlier opinions on the nature of the carotid body were most disparate. At the beginning of this century, however, the view crystallized out that the carotid body was derived from the sympathetic nervous system, its cells of origin being derived by differentiation from sympathetic neuroblasts and identical in nature with the cells from which the medullae of the adrenal glands are derived. In view of this opinion on its origin, the cells of the carotid body came to be considered equivalent to the adrenal medullary cells and exactly comparable in status to other aberrant cells of this nature, such as those which form the organ of Zuckerkandl and similar small isolated cellular groups situated in the ganglia of the sympathetic chain. Thus Kohn (1900) included the carotid body within his category of "paraganglia", a term introduced by him to designate the extramedullary chromaffin tissue associated with the peripheral sympathetic ganglia. Indeed Kohn named the carotid body the "paraganglion intercaroticum", and his interpretation was widely accepted.

Subsequent work showed that this interpretation required some emendation and amplification. In the first place there are striking differences between the known physiological functions of the adrenal medulla and those of the carotid body. The former is an endocrine gland and possesses no known afferent nerve supply. On the other hand, the carotid body has a rich sensory nerve supply, is known to act as a chemoreceptor and has no established endocrine function. Moreover, the adrenal medullary cells, with appropriate methods give a striking chromaffin reaction, whereas those of the carotid body in general show a much less convincing colour change when treated with such mild oxidizing agents as potassium bichromate or potassium iodate. There is, too, an embryological difficulty in the way of accepting a complete identity between the medullary cells and those of the carotid body. The medullary cells can be traced into the developing adrenal glands from the abdominal sympathetic plexuses. In the developmental history of the carotid body, however, the cells of nervous origin that migrate to the mesoderm round the commencement of the internal carotid artery are derived from the glossopharyngeal nerve and not from the cervical sympathetic chain. This glossopharyngeal contribution certainly exists in the development of man (Boyd, 1937) and, in a recent contribution based on extensive material, Batten (1960) has extended the analysis of the origin of these glossopharyngeal cells back to somite stages in sheep embryos. Batten concludes that the initial mesenchymal condensation forming the primordium of the carotid body is augmented by cells which migrate along the carotid branch of the glossopharyngeal nerve, and which are derived in part from the epibranchial placode of this nerve and in part from cells of the petrosal ganglion. From functional, histological and embryological evidence, therefore, it can be concluded that an identification of the carotid body as merely an aberrant portion of chromaffin tissue, completely homologous with the constituent cells of the adrenal medulla, is not acceptable.

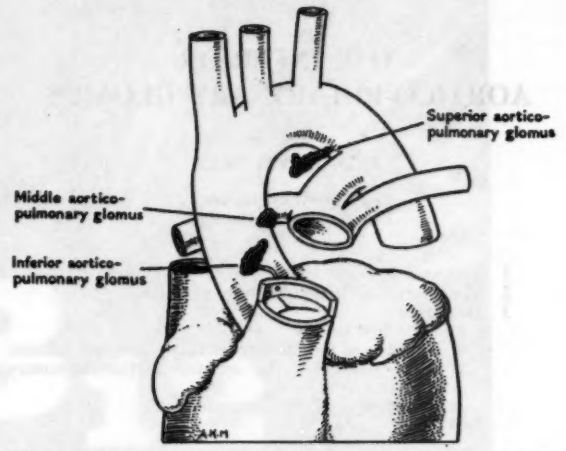
Notwithstanding this conclusion, however, a study of the cells of the carotid body with the electron microscope (Lever & Boyd, 1957; Lever, Lewis & Boyd, 1959) shows that, in the rabbit and the cat, their cytoplasm contains dense membrane-bound granules ranging between 0.05 μ and 0.15 μ in diameter. Though smaller, these granules bear striking resemblances to those found in the adrenal medullary cells (Lever, 1955). Moreover, after fixation by intravital perfusion, it was found possible to obtain staining reactions (chromaffin, diazonium and Giemsa's ferric-ferricyanide) indicative of the presence of phenolic amines in the carotid body cells. Some of the cells of the carotid body also show a marked argyrophil (argentophil) reaction in their cytoplasm when they are stained, for example, with Bodian's method (de Kock, 1954; Willis & Tange, 1959; Boyd, 1961). This silver reaction is also indicative of the presence of catechol amines. In view of the electron-microscopic observations and of the staining reactions shown by the cytoplasm of some of the cells, Lever *et al.* (1959) considered that the membrane-bound granules contain a phenolic amine, and that the available evidence appears to be more in favour of its being adrenaline or noradrenaline, rather than 5-hydroxytryptamine. Our

tentative view, therefore, is that there is included in the mechanism underlying the chemoreceptor function of the carotid body a local humoral factor, which may well be noradrenaline; when released from the cells this, or a related, catechol amine, may induce the discharge of impulses in the afferent fibres of Hering's nerve by stimulating the glossopharyngeal sensory endings in the carotid body. As the term "chromaffin" was introduced to describe the adrenal medullary cells which possess a general endocrine effect, it is a moot point whether the term should also be used for these carotid body cells that show the histochemical reactions for catechol amines. The chromaffin reaction obtained in the carotid body cells is much less definite than that found in the adrenal medulla. Moreover, the carotid body is an afferent mechanism and there is no good evidence to suggest that the catechol amine in its cells is discharged into the general circulation; and, to my knowledge, no one has described an increased urinary catechol content in cases with carotid body tumours. It would seem, therefore, that in the carotid body the cells which give the chromaffin reaction are only a component in an organ system; to classify them in precisely the same category as the cells of the adrenal medulla is to ignore the striking differences between the two cellular groups.

Much less is known about the detailed histology of the structures in the thorax which resemble the carotid body. As will be seen later, however, the available knowledge of their microscopic structure indicates that they, also, possess cells that contain catechol amines. The same terminological problem arises, therefore, as to whether or not they should be classified as chromaffin organs. As Kohn's term "paraganglion" implies a close relationship to the adrenal medulla, it, likewise, is not completely satisfactory for the carotid body and the other chemoreceptor mechanisms (Boyd, 1937). In the present state of our knowledge it may, perhaps, be better to use non-committal terms such as "body" or "glomus" for these chemoreceptors rather than to include them within categories in which they must constitute special cases. Muratori (1959) has suggested that they should be called "paraganglia with a sensory innervation". Such a term seems to me to give the wrong emphasis; it is the sensory element which, surely, is of paramount importance. Indeed, this importance is generally recognized by the wide use of "chemoreceptor" in the description of these small organs. If it is felt necessary to retain the concept of paraganglion in relation to these structures, it should be understood that the paraganglionic tissue is only one element in their composition.

The second terminological difficulty posed by the intrathoracic chemoreceptor homologues of the carotid body is that of selecting names for them that relate them to their topographical situations. The carotid bodies themselves are closely associated with the carotid sinus. This baroreceptor, or pressor-receptor, segment of the internal carotid artery is situated on a vessel that represents the third branchial arch artery of the early embryonic vascular system (Koch, 1931; Boyd, 1934, 1937; Muratori, 1935). In a number of mammals there are small nodules of tissue, closely resembling the carotid body in structure, which are situated in the terminal course of the depressor nerves, or their equivalents. These structures are closely associated with the derivatives of the fourth branchial arch arteries and can, therefore, be conveniently designated aortic arch bodies or glomera. One (the right aortic arch body—the subclavian glomus of Muratori) is situated near the origin of the right subclavian artery, and

FIG. 1. The Aortico-Pulmonary Bodies in a Six-Month Foetus



The inferior body is vascularized by a branch of the pulmonary trunk.

the other (the left aortic arch body) lies close to the aortic arch. There are, so far, no published observations on these aortic arch bodies in man. I have, however, been able to find the left body in several human foetuses, but have been unable to identify it in postnatal specimens. The right aortic arch body in man has so far eluded me, though the human innominate-subclavian arterial arch possesses a baroreceptor area (Boyd, 1950).

These aortic arch bodies, however, are very small. Much more impressive and, presumably, more important functionally, are the larger structures with close resemblances to the carotid body, which are situated more caudally in the thoracic cavity proper. These chemoreceptor organs have been called by a number of different names. Muratori (1959) has suggested the term "aortico-pulmonary paraganglia" for them; I prefer, for the reasons argued above, to name them the aortico-pulmonary bodies or glomera. In arrangement and distribution they show considerable variation in the different mammals in which they have been investigated. In man they fall into three principal groups (fig. 1). There is, firstly, a superior aortico-pulmonary glomus which is situated in the interval between the caudal surface of the aortic arch and the ligamentum arteriosum or, in the foetus, the ductus arteriosus. The relationship of this glomus to the latter vessel explains why Muratori has called it the "paraganglio botallico". Secondly, there is a middle aortico-pulmonary glomus, situated between the ascending aorta and the pulmonary trunk at the level of the bifurcation of the latter vessel. Finally, and nearer the heart, there is the inferior aortico-pulmonary glomus; this is also situated between the two derivatives of the truncus arteriosus, the ascending aorta and the pulmonary trunk. It is with this last of the aortico-pulmonary glomera that the remainder of this contribution is concerned.

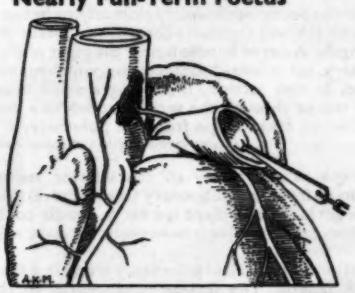
2. The Inferior Aortico-Pulmonary Glomus

As long ago as 1902 Wiesel described the presence of chromaffin tissue near the origin of the left coronary artery in man. He considered that the total amount of such tissue

was as large as, if not larger than, that represented by the medullae of the adrenal glands. A number of subsequent investigators have found this tissue. None of them, however, has described it as constituting anything like so large a mass as indicated by Wiesel, and they have varied widely in their interpretation of the tissue. Thus Trinci (1907), like Wiesel, considered the tissue to be chromaffin, whereas Busacchi (1912), who first indicated the presence of two separate collections of the cells—a superior and an inferior—described the upper structure to possess a very poor chromaffin reaction, and was unable to find such a reaction in the other. More recent investigations (Penitschka, 1931; Palme, 1934; Seto, 1935; Watzka, 1943) have resulted in further descriptions of this juxta-cardiac tissue, but there is still uncertainty about the presence or absence of the chromaffin reaction.

In my collection of human embryonic and foetal material, I can first identify the inferior aortico-pulmonary glomus in specimens of between 20- and 30-mm. crown-rump (C-R) length (50–60 days). The primordium consists of darkly staining cells, closely resembling neuroblasts. These appear to have accompanied the nerves which grow into the loose cellular tissue that becomes established between the aorta and the pulmonary trunk, as a consequence of their separation by the fusion of the spiral septa which divide the truncus arteriosus and bulbus cordis into two distinct vascular channels. These darkly staining cells are more closely related to the pulmonary trunk than to the aorta. There is no specific indication of a mesodermal condensation in the wall of the pulmonary trunk, but very soon the cells of the primordium of the glomus are vascularized by a direct, and striking, branch from the trunk. This artery to the glomus will persist until full term, but in later foetal stages it comes to be supplemented by branches from the ramifications (vasa vasorum) of the coronary arteries on the walls of the aorta and the pulmonary trunk. Usually it is the left coronary artery that provides the main reinforcement to the arterial supply of the glomus. On several occasions, however, I have observed an additional supply from the root of the right coronary artery (fig. 2). After birth, the supply to the glomus from the pulmonary trunk appears to retrogress completely. Certainly I have been unable to find it in the adult, or in a few specimens in the first few months of postnatal life. As the pulmonary branch can be found at all stages until full term, however, it would seem that the disappearance of the pulmonary branch to the glomus must be included among the structural alterations that occur at, or about, the time of birth. The artery from the pulmonary trunk to the glomus is richly innervated. In some embryos fibres can even be traced along its course through the thick wall of the parent vessel (Plate VII: J); this is an appearance which I do not recollect ever having seen elsewhere in the vascular system. There is not, however, any indication that the parts of the walls of the pulmonary trunk, or of the aorta, adjacent to this glomus, possess a specialized baroreceptor apparatus.

FIG. 2. The Inferior Aortico-Pulmonary Body in a Nearly Full-Term Foetus



In addition to the artery from the pulmonary trunk, branches from each coronary artery pass to the glomus. The pulmonary trunk is pulled downwards in this figure.

From this richly vascularized primordium the glomus gradually differentiates into tissue (Plate VII: A) which, in routinely stained material, can in no histological feature be distinguished from the carotid body. In material treated specifically to show nerve fibres, the glomus again shows an identity in appearance with that of the carotid body (Plate VII: B–E). There is a rich periglomeral nerve plexus to which vagal and sympathetic fibres can be traced. From this plexus small nerve bundles pass into the glomus to surround, and help to define, the small lobules which are so characteristic of its established structure. Branches from these bundles terminate, in a complicated plexiform arrangement, on the cells of the glomus and on the arterial branches contained within it. These nervous relations are precisely those found in the carotid body. In human material treated appropriately, I have been unable to persuade myself that a chromaffin reaction, such as is found in the adrenal medulla, is present in the cells of the glomus. There is a suggestion of a yellowish tinge but nothing more. However, in material stained with the Bodian technique there is a most striking display of argyrophilia in many of the constituent cells (Plate VIII: K–P). Similar argyrophilia is shown (see Boyd, 1961, for discussion) by cells in the carotid body, in the adrenal medulla and in the organs of Zuckerkandl; it is also shown by the enterochromaffin cells in the gut. In the inferior aortico-pulmonary glomus the argyrophilic cells are extensively branched and often tend to be grouped round the intra-glomic arteries. Occasionally, indeed, they can be found round the artery of supply to the glomus at some distance from the organ itself. There is a close association of the argyrophilic cells with the nerve fibres in the glomus. Owing to the density of the reduced silver in them and to the branching of their cytoplasmic processes, however, it has not been possible to determine if endings of these nerve fibres actually terminate in relation to the surfaces of these cells. The marked argyrophilia of the cells makes it very easy to identify the glomus in sections. The ability to reduce silver in this striking fashion becomes apparent in the cells of the glomus comparatively early in development. Thus it is present in a specimen of 40-mm. C-R length, with an intensity similar to that obtained in late foetal and in neonatal stages.

3. Discussion

Like the carotid body, the human inferior aortico-pulmonary body consists of glomus cells arranged, in a lobular fashion, round a whorled system of blood vessels. To the resulting complicated mixture of these glomus and vascular cells there is added a very rich nerve supply. All three components of the chemoreceptor mechanism constituted by these bodies require some further discussion.

a. The Blood Vessels

Arnold (1865) considered that the blood vessels in the carotid body were capillary loops. De Castro (1926, 1928,

1951) regarded them as sinusoids, and most recent investigators of the blood vessels in the organ (e.g. de Kock, 1954; Hoffman & Birrell, 1958; Muratori, 1959) accept the view that there is a capillary or sinusoidal network interposed between its arterioles and venules. The view of Schumacher (1938) that the carotid body and other "non-chromaffin" paraganglia represent a complex of arteriovenous anastomoses, the glomus cells themselves being none other than modified myoepitheloid cells ("Quellzellen"), has not been generally accepted. Goormaghtigh & Pannier (1939), however, have indicated that, in the cat, the organ of Penitschka (as they call the equivalent in this mammal of the human aortico-pulmonary bodies) is both a complicated arteriovenous anastomosis and a paraganglion. They considered that the paraganglionic cells, the majority of which lack chromaffinity, are implanted on the arteriovenous anastomoses, in the region of their prevenous segment where their wall is reduced to a single endothelial layer. It is not quite clear why Goormaghtigh & Pannier considered the vessels to be arteriovenous anastomoses. It can be stated, however, that the walls of the arterioles of supply to the capillaries of the human inferior aortico-pulmonary body show the afibrillar modification of their smooth muscle cells, such as is found in the peripheral arteriovenous anastomoses. In the embryo and foetus, as has been described above and illustrated in fig. 2 and in Plate VII: F–J, the artery of supply to the structure stems from the pulmonary trunk. Such an origin for this artery was first noted by Palme (1934) who found it in two foetuses and in one new-born child. As, however, he did not find the pulmonary branch in his remaining specimens he suggested that the branch might be a variant and not regularly present. In view of the fact that it is present in all the large number of human foetuses available to me, this suggestion seems unlikely. Nonidez (1936) also indicated the presence of a pulmonary supply to the equivalent organ in the postnatal cat. He initially surmised that such a supply indicated that the relatively deoxygenated blood of the pulmonary artery was thus provided with a chemoreceptor mechanism. However, the work of Goormaghtigh & Pannier (1939) demonstrated that, in fact, in the adult cat the organ is not supplied by blood from the pulmonary trunk. Likewise, in the inferior aortico-pulmonary glomus of man, the postnatal organ is irrigated by arterial blood. Why the switch from a pulmonary to an aortic supply should occur in late foetal stages in man is not apparent. Perhaps this chemoreceptor mechanism is "set" at levels of relatively high O_2 saturation and could not act if venous blood reached it; or, such venous blood might cause it to be continuously stimulated. The explanation may reside merely in the fact that, as a functioning organ, the glomus requires, or attracts, a blood supply with a high oxygen content. The carotid body itself shows an alteration in its blood supply during development; initially, its vascularization is from small branches of the internal carotid artery, but these are replaced by an artery which stems from the external carotid, or from the ascending pharyngeal, artery.

b. The Cells of the Inferior Aortico-Pulmonary Glomus

These include cells from two embryological sources, neural and vascular. The neural cells appear, from a study of the development of this glomus, to be derived from neuroblasts that have migrated down the vagus nerves. Moreover, on the analogy of the carotid artery, a vagal derivation for the neural component seems more probable than origin from the sympathetic trunks. These cells of neural origin may be of two varieties, argyrophil and non-argyrophil, as has been suggested by de Kock (1954) for the carotid body. These two, however, may be functional variants of a single type. Although the argyrophil cells are dendritic in nature, they do not resemble nerve cells (many of which can, in fact, be found in close association with the periphery of the organ), and it may be that the glomus cells are more closely related in origin to primitive Schwann cells than to truly neurogenic elements. The silver reduction found in the argyrophil cells is so similar to that found in cells of the carotid body, in the adrenal medullary cells and in enterochromaffin cells, that the presence in them of phenolic amines is most probable. As has been argued for the carotid body (Lever *et al.* 1959), it seems more likely that the amine is adrenaline or noradrenaline than 5-hydroxytryptamine. It also seems likely that the amine in the glomus cells possesses a local humoral rather than a general endocrine significance. However, the silver reduction is found in the cells from early stages and throughout subsequent foetal life. If a phenolic amine is involved in the mechanism of stimulation of chemoreceptors, then there seems to be the structural basis for chemoreception by the inferior aortico-pulmonary glomus during foetal life. In utero, the availability of such a mechanism may be of little importance. Its presence at full term, however, cannot be neglected in a consideration of the initiation and control of pulmonary respiration.

PLATE VII. THE INFERIOR AORTICO-PULMONARY GLOMUS

FIG. A. Section through the inferior aortico-pulmonary glomus in a full-term foetus (Stain: Masson; magnification × 265)

FIG. B. Section through a lobule of the aortico-pulmonary glomus in a full-term foetus, to show nerve fibres (Technique: de Castro; magnification × 380)

FIG. C. Section through a part of the inferior aortico-pulmonary glomus in the first year of life, to show nerve fibres (Technique: de Castro; magnification × 380)

FIG. D. Portion of specimen shown in B at a higher magnification. Note the richness of the nerve fibres (Technique: de Castro; magnification × 700)

FIG. E. Portion of specimen shown in B at a higher magnification (Technique: de Castro; magnification × 835)

FIG. F. Origin, from pulmonary trunk, of artery to inferior aortico-pulmonary glomus in a foetus of 200-mm. crown-rump (C-R) length (magnification × 75)

FIG. G. Inferior aortico-pulmonary glomus lying between pulmonary trunk (above) and aorta (below) in a foetus of 140-mm. C-R length. A nerve bundle lies to the right of the glomus. The artery, cut in longitudinal section, which supplies the glomus and, in this section, is sending a small branch to it, can be traced through the serial sections to a vessel, shown in H, which takes origin from the pulmonary trunk (Stain: haematoxylin and eosin; magnification × 95)

FIG. H. Origin of the artery to the inferior aortico-pulmonary glomus from the pulmonary trunk (above). Between it and the aorta (below) there is a nerve bundle containing a small micro-ganglion (Stain: haematoxylin and eosin; magnification × 95)

FIG. J. Section across wall of pulmonary trunk in a foetus of 48-mm. C-R length. The section is tangential to the intramural course of the artery to the inferior aortico-pulmonary glomus. Note the nerve fibres marking out the pathway of this artery (Technique: de Castro; magnification × 465)

THE INFERIOR AORTICO-PULMONARY GLOMUS

(FIGS. A–P)

J. D. Boyd

(page 127)

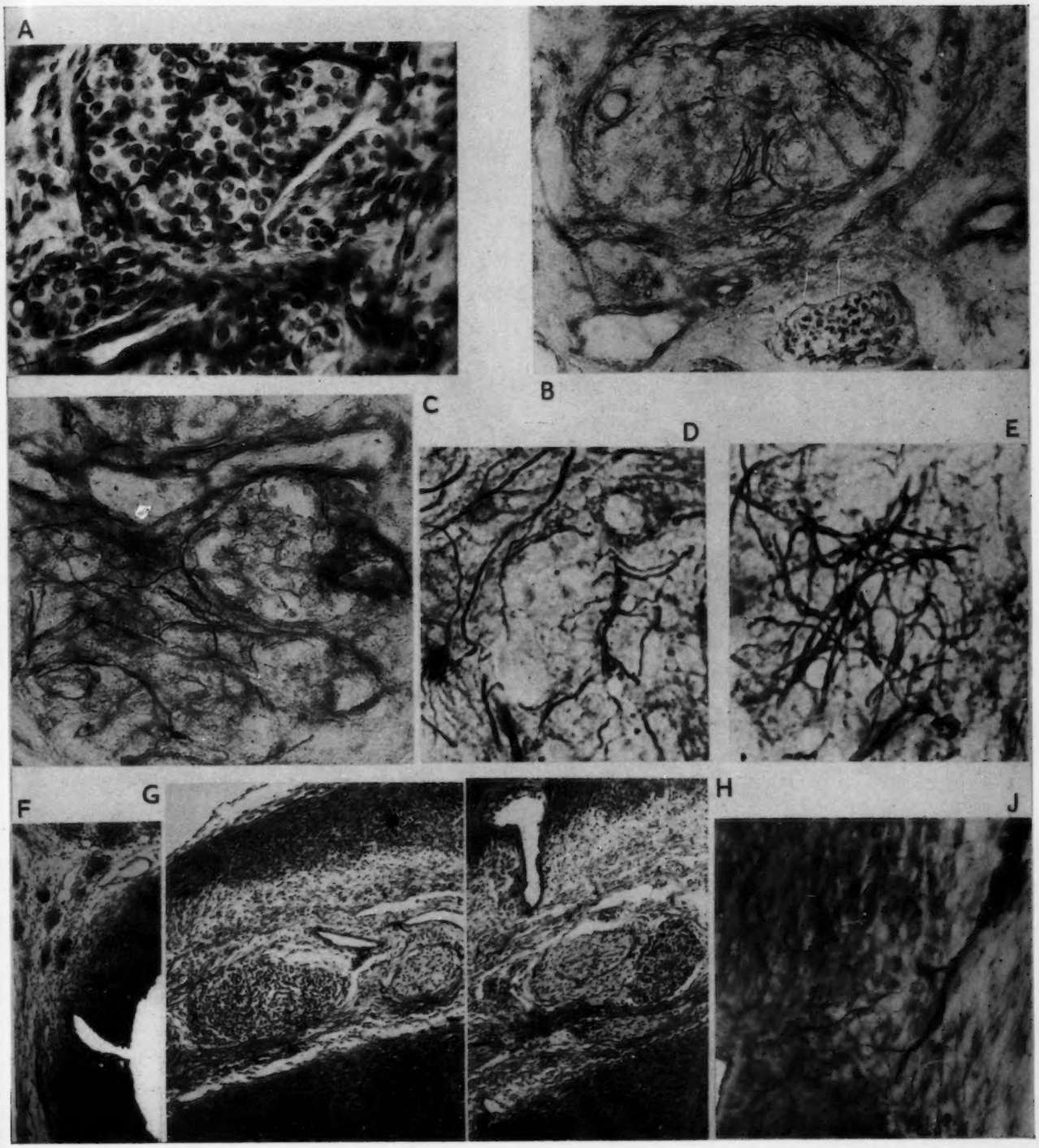


PLATE VIII

THE INFERIOR AORTICO-PULMONARY GLOMUS

(continued)

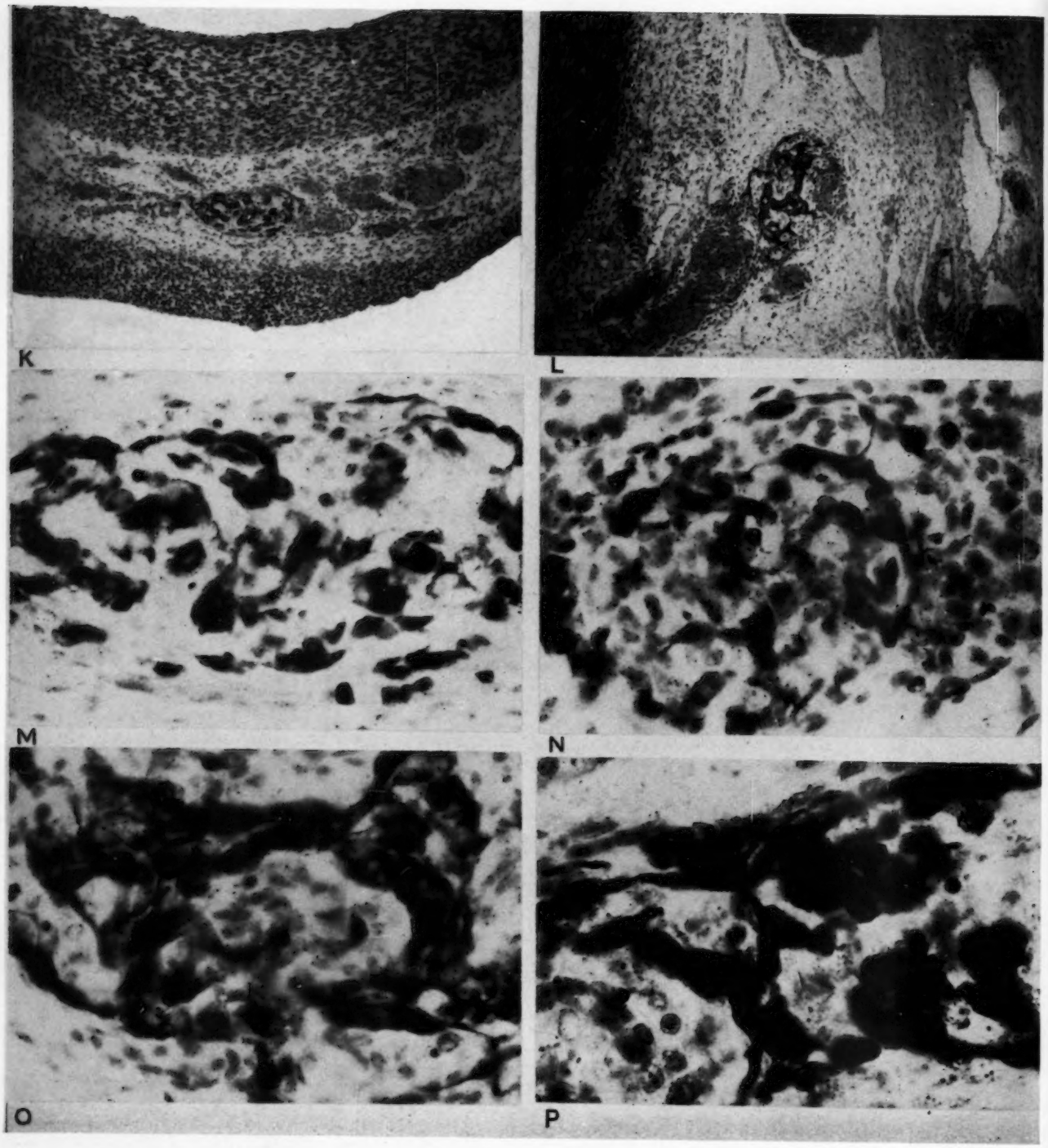


PLATE VIII. THE INFERIOR AORTICO-PULMONARY GLOMUS

FIG. K. Photomicrograph of section through aorta (above) and pulmonary artery (below) at the level of the inferior aortico-pulmonary glomus in a foetus of 100-mm. C-R length. Note the argyrophilia of the glomus and the adjacent nerve bundles (Stain: Bodian; magnification × 85)

FIG. L. Photomicrograph of section at level of superior aortico-pulmonary glomus in a foetus of 100-mm. C-R length, for comparison with K. This glomus also contains a markedly argyrophilic component and is closely related to vago-sympathetic nerve bundles (Stain: Bodian; magnification × 85)

FIG. M. Photomicrograph of section through inferior aortico-pulmonary glomus in a 100-mm. human foetus, to show distribution of branching argyrophilic cells (Stain: Bodian; magnification × 550)

FIG. N. Photomicrograph of section through inferior aortico-pulmonary glomus in a 120-mm. human foetus. Note the branching argyrophilic and the non-argyrophilic cells (Stain: Bodian; magnification × 550)

FIG. O. Argyrophilic and non-argyrophilic cells in the inferior aortico-pulmonary glomus of a nearly mature foetus (Stain: Bodian; magnification × 525)

FIG. P. Argyrophilic and non-argyrophilic cells in the inferior aortico-pulmonary glomus of a full-term foetus. Note the extensive ramifications of the argyrophilic cells (Stain: Bodian; magnification × 550)

The cells of vascular origin in the glomus are those constituting the endothelial lining of the capillaries and their adventitial derivatives, the pericytes. The latter may be concerned in the chemoreception mechanism; they may, however, be no more than a constituent part of the connective tissue of the organ.

c. Nerve Supply to the Inferior Aortico-Pulmonary Glomus

This is derived from the conjoined bundles of vagal and sympathetic fibres which pass towards the heart between the aorta and the pulmonary trunk. In silver-stained material the fibres are of widely varying calibres, suggesting an extensive spectrum of fibre diameter. In the absence of specimens stained for myelin, I am, however, unable to give significant details. In addition to nerve-endings related to the glomus cells there is a rich nerve supply to the arterioles in the organ. As has already been described, this supply extends along its main nutrient artery.

d. Distribution of Chemoreceptors

A comparison of the structure of the human inferior aortico-pulmonary glomus with the carotid body and with homologous structures in other mammals shows such detailed resemblance that an identity of function can reasonably be expected. The available physiological evidence (see Heymans & Neil, 1958, for discussion) strongly supports this expectation. There is, however, much variation amongst mammals in the precise sites in which the homologues of the carotid body are found. Muratori (1959), who gives a full account of the variation, has recently strongly supported the association suggested by Koch (1931), Boyd (1934) and himself (1935) between the position of chemoreceptors and areas of arteries which not only possess a baroreceptor mechanism, but represent persistent parts of the embryonic and phylogenetic branchial arch arterial pattern. Such an association does not hold for the inferior aortico-pulmonary glomus. This structure is, however, closely related to the stem of origin of all the arch arteries, the truncus arteriosus. What the relationship may be between the chemoreceptor organs and certain structures such as the "non-chromaffin paraganglia" of the vagus nerve or the glomus jugulare is not yet apparent. These structures have not been shown to possess an afferent nerve supply. Nevertheless, in an electron-microscopic study of a glomus jugulare tumour it has been shown (Boyd, Lever & Griffith, 1959) that the tumour cells possess osmiophilic membrane-bound granules which overlap in size those of the carotid body. Other features in the cytology of the tumour also indicate a distinct resemblance between the two structures. There is, however, no evidence to indicate the possession by the glomus jugulare of a chemoreceptor function.

* * *

The inferior aortico-pulmonary glomus is described and its relationship with other chemoreceptor mechanisms is discussed. Attention is drawn to its blood supply which, in the foetus, is derived from the pulmonary trunk. The presence of a system of argyrophil cells in the glomus is described. It is suggested that the argyrophilia is due to the presence in the cells of catechol amines, which may be concerned in the chemoreceptor mechanism. The argyrophilia is present from very early stages of foetal life, which suggests that the glomus may have a function in this period.

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MINERAL METABOLISM OF THE FOETUS AND NEW-BORN

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1 Development and composition
2 Mineral metabolism of the foetus
3 Mineral metabolism of the new-born animal
References

1. Development and Composition

For the whole of its life, before birth and afterwards, the animal body has within it a variety of different inorganic elements, most of which are vital to its existence. Some ten years ago the mineral metabolism of the foetus and new-born was discussed from the incremental point of view (Widdowson, 1950; McCance & Widdowson, 1951; Widdowson & Spray, 1951). This still remains the fundamental approach, and there is no need to modify anything that was written then, but since that time further analyses have been made, and our knowledge at present is set out from rather a new angle in Table I. The great general principle brought out by a study of this table is that development both before and after birth is not just a matter of an increase in size. It also involves a change in composition. Thus, if a foetus weighing 175 g. were to increase 20 times in size it would weigh 3.5 kg., but it would not have the composition of a full-term baby; and were the full-term baby to multiply itself 20 times it would weigh as much as a 70-kg. man, but it would not have the same composition. Owing to the steady fall throughout development in the amount of water and extracellular fluids in the body, mere increase in size both before and after birth would give a product containing too much water and too many chloride ions. Against this, normal development involves the deposition of more nitrogen, potassium and magnesium than would be called for by increase in size alone, and this is because of the increase in cell substance in all parts of the body. The outstanding example of this relationship is the great increase in the deposition of calcium, and to a lesser extent of phosphorus, over and above the amounts called for by size alone. This is due to the maturation and with it the calcification of the bones—tissues which hardly exist in early foetal life, and can have little or no function until after birth. As the bones become calcified, magnesium and sodium are also incorporated into their structure and this explains why the amount of sodium per kg. does not fall so much as the amount of chloride, and why the magnesium rises more than the potassium.

Owing to the increase of structural cellular material throughout foetal life at the expense of watery extracellular fluid, the composition of foetal tissues may differ materially from that of adults. This is well illustrated by Table II (Dickerson & Widdowson, 1960), which shows the composition of skeletal muscle in immature foetuses, new-born infants and adults, of two different species. In each of them the solids—potassium, magnesium and phosphorus—in the muscles of the foetus were well below those in the muscles of the adult, and for the reasons just given. The high concentrations of calcium, however, were probably intracellular and could not be explained in the same way. They were attributed to the high proportion of nuclei in the foetal muscle cell and the high concentrations of calcium therein. Other examples of the effect of growth and development on structure have been given by Dickerson (1960) and Widdowson & Dickerson (1960).

2. Mineral Metabolism of the Foetus

Ion transport, sodium pumps and potassium–hydrogen exchange mechanisms have so dominated physiological thinking in the last few years that it is difficult to carry one's mind back to the days when so much was regulated, or thought to be regulated, by diffusion. It goes without saying that the

TABLE I. Changes Taking Place in Development, Set Off Against Those Due to a Simple Increase in Size

	Water (kg.)	Total N (g.)	Na (m-equiv.)	K (m-equiv.)	Cl (m-equiv.)	Ca (g.)	Mg (g.)	P (g.)	Fe (mg.)	Cu (mg.)	Zn (mg.)
A foetus weighing 175 g. contains	0.154	2.45	18.2	7.4	13.7	0.60	0.024	0.42	9.25	0.575	3.50
This foetus × 20 would contain	3.08	49	364	148	274	12.0	0.48	8.4	185	11.5	70
A baby weighing 3.5 kg. contains	2.4	66	243	150	160	28.2	0.76	16.2	262	13.7	53
This baby × 20 would contain	48	1,320	4,860	3,000	3,200	564	15.2	324	5,240	274	1,060
A man weighing 70 kg. contains	42	2,000	5,150	4,050	2,940	1,320	27.4	740	4,350	100	1,640

TABLE II. Effect of Development on the Composition of Skeletal Muscle of Man and the Pig

(Values expressed per kg. fresh muscle)

	Water (g.)	Total N (g.)	Na (m-equiv.)	K (m-equiv.)	Cl (m-equiv.)	Ca (m-equiv.)	Mg (m-equiv.)	P (m-mole)
Man:								
Foetus, 20 weeks	887	14.8	90.6	57.6	65.6	7.1	10.5	40.0
New-born	804	20.7	60.1	57.7	42.6	4.3	14.8	47.0
Adult	790	30.8	36.3	92.2	22.1	2.6	16.7	58.8
Pig:								
Foetus, 46 days	916	8.5	106	46.7	72.7	7.3	9.0	30.7
New-born	823	15.4	54.3	72.5	36.8	2.9	15.3	56.8
Adult	735	31.1	24.2	92.5	21.1	2.3	23.2	68.0

principles of active transport which control the mineral composition of an adult's cells are likely to apply also to those of the foetus. The sources of energy, however, may not necessarily be the same. It has recently been shown, for instance (Whittam, 1960; Capek, 1961), that the foetal kidney can utilize the energy derived from anaerobic metabolism to maintain the mineral integrity of its cells, whereas that of the adult cannot. This is entirely in keeping with the accepted ability of foetal and new-born animals to survive much longer periods of oxygen deficiency than their parents.

Many investigators have compared the concentrations of sodium, potassium, calcium, and magnesium in foetal and maternal plasma, and these are highly suggestive both as regards ion transport and the mineral metabolism of the foetus. There is general agreement (see Smith, 1959) that foetal plasma usually contains more calcium and magnesium and certainly more phosphorus than maternal plasma. This has been demonstrated in pigs, cows and rabbits as well as in man; and a further, and highly significant, fact has been added, namely that in cattle (Sato, 1933) and in man (Hallman & Salmi, 1953; Salmi, 1954) the concentration of calcium in the umbilical vein exceeds that in the umbilical artery. It may be inferred that some of this calcium reaches the umbilical vein by active transport through the placenta, because the concentrations of calcium in the maternal sera have been found to be below those in the umbilical vessels, and the placenta, if not over-mature and becoming pathologically calcified, contains negligible amounts (Widdowson & Spray, 1951).

The concentrations of potassium have also been found to be higher in foetal than in maternal plasma (Widdowson & McCance, 1956; Westin, Kaiser, Lind, Nyberg & Teger-Nilsson, 1959), and reasoning similar to the above suggests that the maintenance of these concentrations must be the result of active transport. By the time blood has been withdrawn, however, the foetus is generally already suffering from an oxygen deficiency, and it has been suggested (James, personal communication, 1958) that the high serum potassium concentrations found in the foetus, and sometimes in the premature and full-term baby, have been due to anoxaemia, since dangerously high concentrations of potassium have been found in the sera of hypoxic human infants suffering from respiratory distress. This criticism cannot be made of the high concentrations of potassium found in the sera of normal new-born pigs. Salmi (1954), moreover, was alive to this danger in all her work on calcium and showed that, although experimental hypoxia did raise the concentration of calcium in the serum, her conclusions were not upset by it. She showed furthermore that the concentrations of magnesium, frequently found to be higher in foetal than in maternal serum, were not raised by low oxygen tensions.

The work carried out with radioactive isotopes gives results which are in agreement with the above general hypothesis (Shirley, Jeter, Feaster, McCall, Outler & Davis, 1954; Feaster, Hansard, Outler & Davis, 1956). Thyroxine is not transferred across the placenta, but the concentration of inorganic iodine may be five times higher in the foetal than it is in the maternal plasma towards the end of pregnancy in rabbits (Logothetopoulos & Scott, 1955; Hall & Myant, 1956; Friis & Hall, 1957). Fuchs & Fuchs (1957a, 1957b) showed that injected phosphorus passed much more rapidly into the foetus against a concentration gradient than it did in the reverse direction, and Wilde, Cowie & Flexner (1946) had previously concluded that all the phosphorus reaching the foetus was trapped there by the growth of soft tissues and bone. There is also evidence from other directions (see section 3) that the supply of phosphorus may govern the rate of growth both of the foetus and the newly born. As regards water (Plentl, 1958) and most other elements, this is certainly not so, for Wasserman, Comar, Nold & Lengemann (1957) found that nearly twice as much calcium was transferred to the foetus than the foetus required for growth. All the iron actively transferred to the foetal rabbit has also been shown to remain there, but in this case the supply is greater than the immediate demands of growth and most of the iron is deposited in an inorganic state (Bothwell, Pribilla, Mebust & Finch, 1958).

The mineral structure of foetal membranes and fluids has been examined by Alexander, Nixon, Widdas & Wohlzogen (1958), Davies (1958), Economou-Mavrou & McCance (1958), McCance & Stanier (1960), McCance & Widdowson (1960) and Stanier (1960). There would appear to be a rapid build-up of calcium in the pig's chorio-allantoic membrane between the 20th and 46th day of gestation, with subsequent decline to much lower concentrations as the rate at which calcium is deposited in the foetal bones goes up. In the light of the evidence just quoted it is reasonable to suppose that this calcium does not reach the inside of the membrane solely by diffusion, but at present there is no proof of this.

All are in agreement that the allantoic fluid contains less sodium and chloride than the maternal plasma, and is frequently hypotonic in consequence. This is particularly so in the pig, and McCance & Widdowson (1960) added further facts about this fluid, namely, that by the 46th day of gestation (gestation period, 120 days) it has a pH of about 6, largely

owing to carbonic acid, and that it contains ammonia. The allantoic fluid is often regarded as the product of the foetal kidney, but in the sheep it frequently contains less sodium and chloride than urine collected soon afterwards (Alexander *et al.* 1958), and at the 46th day of gestation the mesonephros of the foetal pig seems to produce a fluid containing as much chloride as the plasma (Stanier, 1960). Crawford & McCance (1960), however, have found an active sodium transport mechanism in the pig's chorio-allantoic membrane, which transfers sodium actively from the fluid to the endometrial surface. The process is self-regulated in that it is inhibited by a fall in pH, particularly if this is caused by carbonic acid, and by the presence of some substance not yet identified in the allantoic fluid itself. If some such system as this operates in the placentas of other species it may help us to unravel some of the knotty problems of mineral metabolism before birth.

3. Mineral Metabolism of the New-Born Animal

The human baby, unlike the new-born of other species, is not provided with a free supply of milk for the first 48 hours of its life. This makes it rather a special case for this period, but thereafter the principles underlying its mineral metabolism closely resemble those which control the metabolism of other species.

All new-born animals have a great capacity for growth, and this dominates the whole of their metabolism. Growth and the composition of the diet are in large measure responsible for the maintenance of a normal internal environment, for the kidney of some new-born animals is so immature that it can keep the proportions of water, sodium, and potassium within normal limits only if growth is unrestricted and its requirements are being met by a perfect diet.

Smith, Yudkin, Young, Minkowski & Cushman (1949), Hansen & Smith (1953) and McCance & Widdowson (1954, 1955) have studied the metabolism of premature and full-term human infants during the first days of life, and McCance & Widdowson (1956) have supplemented this by an experimental study of fasting new-born pigs. Some loss of weight is inevitable at this time, but it can be reduced by the administration of water, and still more by water and glucose. The loss of weight is due to the expenditure of glycogen, fat and tissue protein to maintain life, and the products of metabolism are either excreted or retained. The ratio of nitrogen to potassium in the urine is, however, only half that of adults subjected to comparable degrees of food and water deficiencies. The breakdown of tissue protein is exceedingly small at this time of life (McCance & Strangeways, 1954), for the balance between anabolism and catabolism is weighted more strongly in favour of the former than it is in adults. More potassium, however, is excreted during starvation than is called for by the breakdown of tissue protein during the first two days of life, and much more potassium is retained as soon as the infant begins to receive adequate amounts of breast milk. The movements of potassium may reflect movements of glycogen. The excretion of sodium and chloride by the normal infant is not large in view of the amount of extracellular fluid in the body, but it may be considerable if the infant is oedematous and becoming less so. The volume of urine depends upon whether additional water is being administered or not, and is regulated, if no water is being given, by the amount of osmolar material being eliminated by the kidney. The latter is not affected by additional water (Hansen & Smith, 1953).

It has already been suggested that the rate of growth of the foetus may depend upon the amount of phosphorus reaching it, and the human infant normally excretes practically no phosphorus during the first 24 hours of its life. In spite of being given no food, it begins to excrete phosphates during the next 24 hours in response to a rise in the concentration of inorganic phosphate in the serum. This is due to the breakdown of glycogen and tissue protein, necessitated by the absence of food, for by the 7th day, when the intake of phosphorus from breast milk has risen to about 25 mg./kg./24 hr., the serum phosphorus has fallen to its level at birth, and phosphates have disappeared from the urine. All aspects of this syndrome can be reproduced in new-born pigs (Widdowson & McCance, 1959).

Many instances might be given of the dominating influence of food and growth on the electrolyte stability of the new-born animal (McCance, 1959). It has been shown, for instance, that the new-born puppy cannot tolerate the amount of water in a normal supply of milk unless it is accompanied by the milk solids. No cell growth can take place, the kidney is too immature to excrete the water, which is in consequence retained, and the concentrations of electrolytes in the serum and cells are forced down to pathological levels (McCance & Widdowson, 1958a). The converse of this has been demonstrated in infants and in piglets by adding salt to the milk.

Owing to the very large intake of fluid by the new-born animal, quite a small percentage of salt in the milk can lead to the ingestion of more salt per unit of body-weight than an adult would normally take. Given the corresponding amount of water, however, an adult would excrete both the water and the salt without a breakdown in the stability of the body fluids. Not so the human infant. Figure 1 shows the effect of making up the milk for three premature babies so that it contained 0.7% of sodium chloride. In each one the normal gain in weight from day to day altered to a more rapid one, which was in fact pathological. In a short time the babies became oedematous and sick, but on putting them back on to their proper intake of water and salt their weights fell and they began to gain weight again at their previous and more satisfactory rate (McCance & Widdowson, 1957). Similar

FIG. 1. Weight Charts of Three Infants Before, During and After the Period of Salt Administration

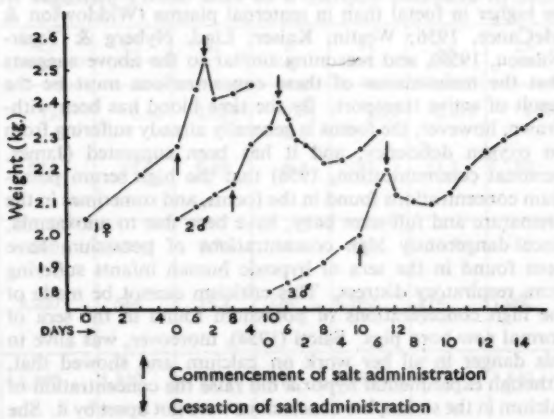

TABLE III. Effects of Giving New-Born Piglets Water or an Equal Volume of Sodium Chloride Solution (0.9 g.% (w/v))

	Water alone	Water+salt
Change of weight in 40 hr. (g./kg. birth-weight)	−53	+96
Percentage of water in bodies	82.7	87.4
Na balance (m-equiv./kg./24 hr.)	−0.3	+35.5
Serum Na (m-equiv./l.)	138	147

results have been obtained by Martínek, Stanichová & Janovský (1961).

Table III shows exactly the same effect in piglets to which no food was given, but only water or salted water. The gain of weight in piglets given the solution of salt without food was quite clearly pathological. Table III, however, shows other features. Firstly, although the amount of water retained in the body was large, it was not large enough to prevent a rise in the concentration of sodium in the serum. This also happened in the babies, and in both species the administration of the salt led to hypertonic expansion of the extracellular fluids.

Our knowledge about mineral metabolism in the suckling period has largely been derived from metabolism experiments but, owing to the technical difficulties involved, very few have been carried out on animals, and most of the information we possess has been derived from studies of the relatively slow-growing human infant, conducted, moreover, after the strictly new-born period. The initial stages of extra-uterine growth remained largely neglected until Widdowson, Slater, Harrison & Sutton (1960) and Slater (1961) set themselves the task of filling in this gap, and in particular of comparing the retentions and mineral balances of infants on breast milk with those on a preparation of cow's milk. These authors studied the infants during the 6th, 7th and 8th days of their lives, and some of their results are shown in Table IV. As might have been expected from a knowledge of the composition of breast milk and cow's milk, the babies on cow's milk were being given considerably more potassium, calcium, strontium, magnesium and phosphorus than the others were getting from their breast milk, and the table shows that they were absorbing and retaining considerably more calcium, strontium, magnesium and phosphorus and nearly twice as much potassium. This was not unexpected, and raises the problem previously voiced by others (Beem and Smith, see McCance & Widdowson, 1958b) as to the fate of the additional potassium retained, for babies fed on these and similar preparations of cow's milk do not retain nitrogen in proportion to potassium, and grow no faster than breast-fed infants.

TABLE IV. Mineral Balances of Infants 6–8 Days Old on Breast Milk and Cow's Milk

(Values expressed per kg. body-wt./24 hr.)

	Na (m-equiv.)	K (m-equiv.)	Ca (mg.)	Mg (mg.)	Sr (μg.)	P (mg.)
Intake:						
Breast milk	2.4	2.3	36	3.5	8	20
Cow's milk	2.5	4.6	135	11	72	103
Net absorption:						
Breast milk	2.2	1.9	20	2.2	−6	18
Cow's milk	2.2	4.0	82	7.0	53	86
Urinary excretion:						
Breast milk	1.7	0.7	4.4	0.6	14	0.5
Cow's milk	2.3	2.0	2.4	0.3	2	35
Retention:						
Breast milk	0.5	1.2	15	1.6	−20	18
Cow's milk	−0.1	2.0	79	6.7	51	51

The additional calcium can find a lodgement only in the bones, and the potassium must go to the soft tissues. From an experimental approach in pigs, McCance & Widdowson (1958b) concluded that the muscles were probably the most important site, but the chemical changes at cell level are quite unknown. There is more evidence about the calcium, strontium and magnesium. It is generally agreed that over the growth period as a whole the skeleton becomes more calcified, but there is evidence that in fast-growing animals, such as kittens (Burns & Henderson, 1936; Weidmann & Rogers, 1958; Slater and Widdowson, unpublished), the calcification of the skeleton does not keep pace with its increase in size over the period of intensely rapid growth during suckling; even in human infants the percentage of calcium in the body may be no higher at six months than it is at birth (Stearns, 1939). There is, therefore, in all probability a place for the additional calcium, phosphorus, magnesium and strontium in the partially calcified osteoid tissues. This process of additional calcification in the early phases of extra-uterine growth is clearly self-limited and must be expected to lead to relatively less mineral deposition later when growth slows down.

A further point of some importance is brought out by Table IV. In spite of the large amounts of calcium, magnesium, and particularly of strontium, absorbed and retained by the babies having cow's milk, less of each of these elements was excreted in the urine. This would be quite in order if the amounts of phosphorus available in breast milk were limiting the deposition of these divalent elements in bone. To prove this, babies fed on breast milk were given a supplement of neutral sodium and potassium phosphates, with the results shown in Table V. These speak for themselves.

The behaviour of strontium, vis-à-vis calcium, has still to be explained. It will be noted that the breast-fed babies were excreting more strontium in the faeces than their food contained, and seven times as much in the urine as the infants on

TABLE V. Effect of Supplementing Breast Milk with Inorganic Phosphate on the Urinary Excretion of Calcium, Magnesium, Strontium and Phosphorus in the New-Born Period

(Values expressed per kg. body-wt./24 hr.)

	Ca (mg.)	Mg (mg.)	Sr (μg.)	P (mg.)
Breast milk unsupplemented	4.4	0.6	14	0.5
Breast milk supplemented with phosphate	2.1	0.2	2.9	20
Cow's milk	2.4	0.3	2.0	35

cow's milk. It is known from the work of Chen & Neuman (1955), Comar, Whitney & Lengemann (1955), Comar (1956) and Wasserman *et al.* (1957) that calcium and strontium compete for the biological transport mechanisms hitherto investigated, and that calcium is preferentially absorbed from the gut, deposited in the bones of the mother and transferred to the foetus. It is also known that there are probably considerable species differences in all this. With phosphorus limiting the rate of mineralization in breast-fed infants, and from what is known about the rapid turnover of minerals, even in an apparently static tissue such as bone, it is easy to see why the babies on breast milk should have been in such large negative strontium balance, although it is not yet possible to fill in all the details.

"... thou knowest not ... how the bones do grow in the womb of her that is with child ..." (*Ecclesiastes*, **11, 5**).

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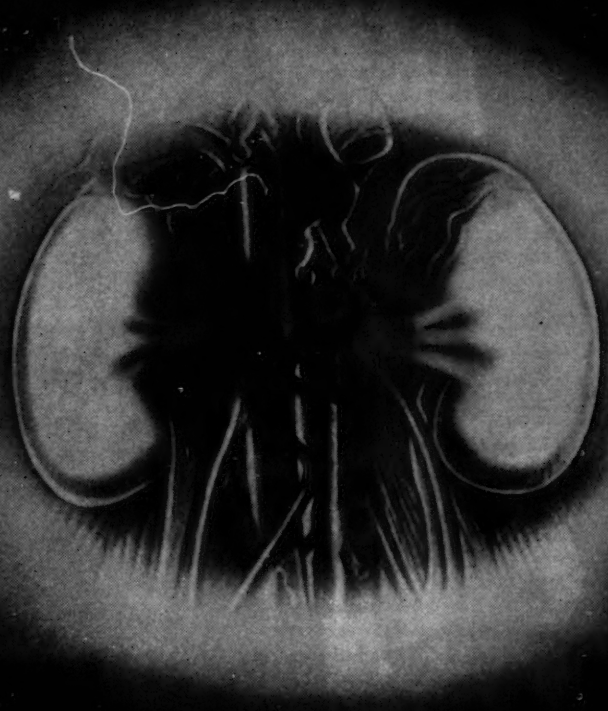

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GLYCOGEN RESERVES AND THEIR CHANGES AT BIRTH AND IN ANOXIA

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1 Tissue glycogen before and after birth
  a Liver
  b Lung
  c Heart
  d Skeletal muscle
  e Other tissues
2 Factors responsible for glycogen formation
3 Factors affecting tissue glycogen
  a Anoxia
  b Nutrition
4 Importance of tissue glycogen
  a To the foetus
  b To the new-born animal
5 Conclusion: the situation with regard to man
References

It is just over one hundred years since Claude Bernard (1859b) isolated glycogen from foetal tissues and described its distribution in foetuses of different ages. He concluded that "Il est permis de penser que chez le foetus cette matière glycogène a un rôle important à remplir dans le développement organique", and by the end of the last century it was generally believed that many embryonic tissues were especially rich in glycogen which was in some way essential for growth and differentiation. Most of this early work was histochemical and, as more accurate methods of quantitative analysis were developed, "This belief died out when it came to be found that glycogen is not present in embryonic tissues to a greater extent than in adult ones" (Needham, 1931). The purpose of this paper is to describe recent quantitative work showing that certain foetal tissues do contain large amounts of glycogen which, though no longer thought to play a specific part in the development of the foetus, may, nevertheless, be of vital importance to both the foetus and the new-born animal. The large body of histochemical work will not be described in detail, since it is in good agreement with the results of chemical analysis, but references to it and to the early quantitative work may be found in reviews by Cremer (1902) and Needham (1931).

1. Tissue Glycogen Before and After Birth

Although there have been some comprehensive histochemical surveys of glycogen distribution in foetal tissues (Bernard, 1859b; Creighton, 1896; Sundberg, 1924), there have been no attempts to make as detailed a quantitative survey, and the data summarized in figs. 1–4 have been taken from a number of different sources. Each figure shows in the left-hand panel the glycogen concentration in foetal tissues, while in the right-hand panel postnatal changes in glycogen concentration are shown. In order to condense data from different species with different lengths of gestation, full gestation is expressed as 100%, and proportions of full gestation as the appropriate percentage; the length of gestation for each species is given in Table I. Whereas the data from other sources are for tissue glycogen, my own data are for tissue total carbohydrate, determined by the method of Kemp & Kits van Heijningen (1954). Histological examination of the tissues confirmed that these values were related to their glycogen content, and figs. 1–4 show that they are of the same order as those for glycogen obtained by other authors. Moreover, good agreement has been obtained between values for total carbohydrate and glycogen in the liver and skeletal muscle of foetal dogs (Schlossmann, 1938) and in the hearts of new-born rats (Stafford, personal communication), and it is therefore probable that the measurement of total carbohydrate gives a reasonable estimate of tissue glycogen (often to within 1%). Estimating total carbohydrate has the advantage that losses due to glycogenolysis are avoided, since its products, down to glucose-1-phosphate and free glucose, are all included in the assay (Mendel, Kemp & Myers, 1954).

TABLE I. Gestation Periods in Different Species

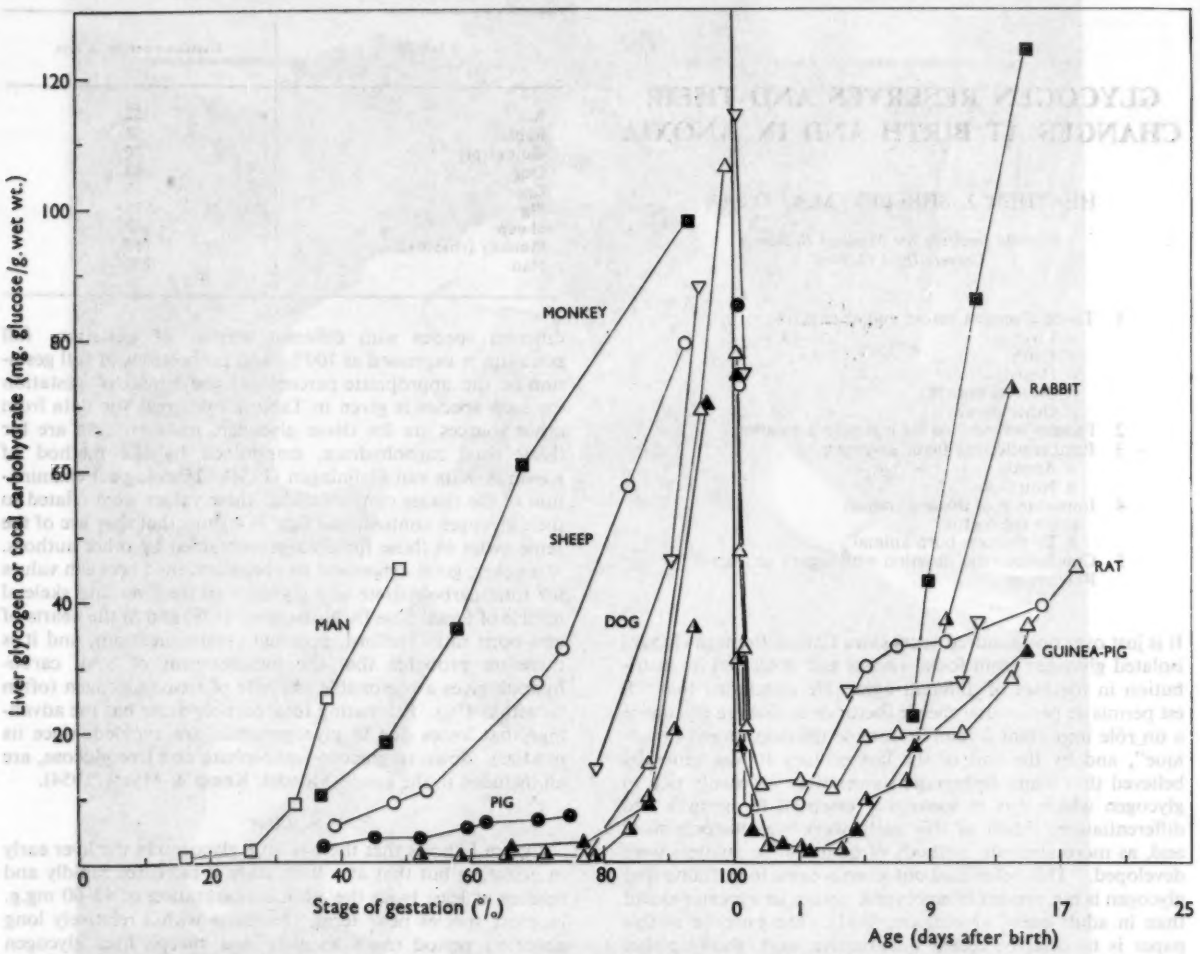
Species	Gestation period in days
Rat	22
Rabbit	31
Guinea-pig	70
Dog	63
Cat	65
Pig	120
Sheep	147
Monkey (rhesus)	168
Man	280

a. Liver

Figure 1 shows that there is little glycogen in the liver early in gestation but that at a later stage it increases rapidly and reaches at least twice the adult concentration of 40–60 mg.g. in most species near term. In those with a relatively long gestation period (man, monkey and sheep), liver glycogen begins to accumulate fairly early, at a steady rate of about 2 mg./g./day in foetal lambs, but where the gestation period is short, and in the pig (Aron, 1922), the rise occurs in the last fifth of gestation and proceeds at the phenomenal rate of 10–20 mg./g./day in foetal rabbits, dogs and guinea-pigs, and 20–40 mg./g./day in foetal rats. Confirmatory data for the low values early in gestation and the stage at which liver glycogen begins to accumulate are provided by Lochhead & Cramer (1908) (rabbit), Aron (1922) (sheep, pig, guinea-pig), Corey (1935a) (rat) and Hard, Reynolds & Winbury (1944) (guinea-pig), but in most of these papers, and in that by Szendi (1936) on man, the values given for foetal liver glycogen near term are lower than those in fig. 1. In the smaller species and in the pig, this might sometimes be accounted for by imprecise knowledge of conceptual age, but it is probable that the analysis of insufficiently fresh material (certainly with regard to man), foetal asphyxia caused by killing or stunning the mother before delivery, and/or inadequate maternal nutrition, were often contributory factors (see under section 3).

After birth the liver glycogen falls very rapidly and usually reaches 10% or less of its initial value within 2–3 hours. This

FIG. 1. Liver Glycogen in Different Species Before and After Birth



The vertical line indicates both term and time of birth.

- □ man (Szendi, 1936; Villee, 1954)
- ■ rhesus monkey (Shelley, 1960, and unpublished data)
- ○ sheep (Shelley, 1960)
- ● pig (Mendel & Leavenworth, 1907; McCance & Widdowson, 1959)
- ▽ dog (Demant, 1887; Schlossmann, 1938)
- △ rat (Stuart & Higgins, 1935; Martínek & Mikuláš, 1954; Jacquot, 1955; Stafford & Weatherall, 1960)
- ▲ rabbit (Szendi, 1936; Jost & Jacquot, 1955; Shelley, unpublished)
- ▲ guinea-pig (Aron, 1922; Shelley, unpublished)

fall occurs even when the new-born animal has suckled within half an hour of birth and is being reared under natural conditions, and the level remains low for several days before gradually rising towards the adult level which is usually reached or exceeded within 2–3 weeks.

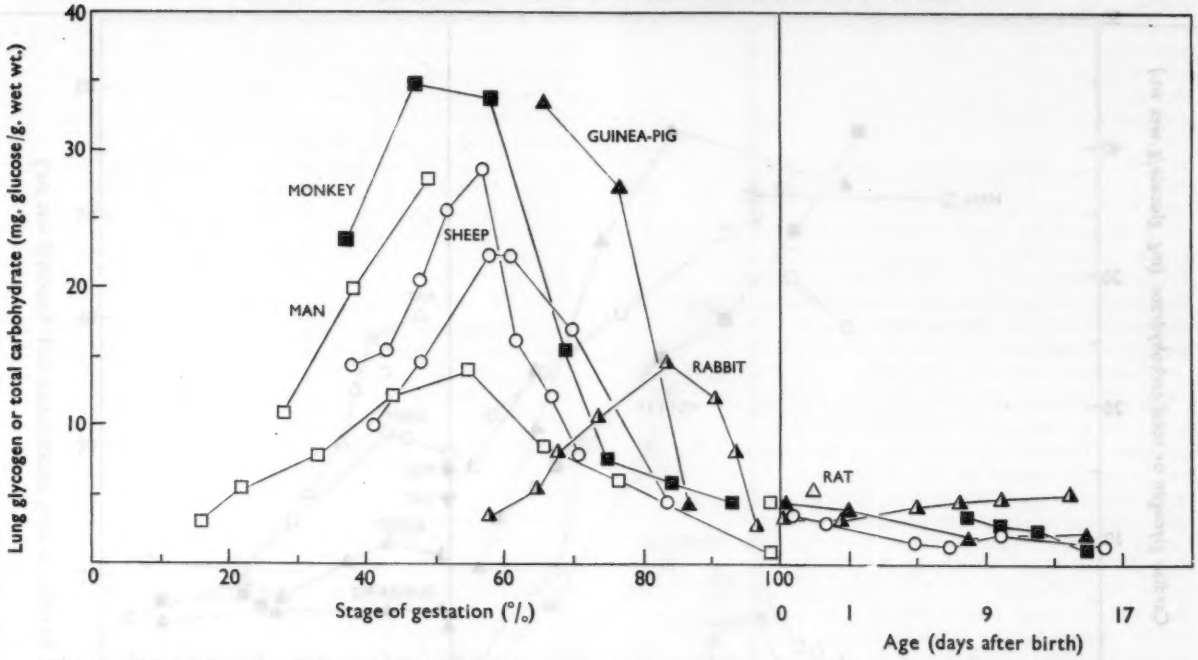
b. Lung

Figure 2 shows that, whereas relatively little glycogen is present in foetal lungs early in gestation, this later rises to peak values of up to 34 mg./g. and subsequently falls to near-adult levels of less than 5 mg./g. before birth, after which there is little change. Two sets of results for sheep are shown because these illustrate the variation encountered within the species (Shelley, 1960). Szendi's results for man and the rabbit are low compared with Villee's for man and those for other species, but are included in fig. 2 because they indicate the stage of gestation at which the fall occurs. This, too, is related to the length of the gestation period, since the fall occurs just over half-way through gestation in man, the rhesus monkey and sheep, rather later in the guinea-pig and not until the last fifth of gestation in the rabbit. Histological examination shows that the glycogen is concentrated in the foetal alveolar epithelium, and Fauré-Fremiet & Dragoiu (1923) calculated that in the lamb half-way through gestation this must contain 75–78 mg./g.; the fall in lung glycogen is associated with the loss of this epithelium.

c. Heart

Whereas all the species investigated have high levels of liver glycogen and low levels of lung glycogen at term, regard-

FIG. 2. Lung Glycogen in Different Species Before and After Birth



The vertical line indicates both term and time of birth.

□ man, lower curve (Szendi, 1936)
□ man, upper curve (Villee, 1954)
■ rhesus monkey (Shelley, 1960, and unpublished data)
○ sheep, lower curve (Shelley, 1960)
○ sheep, upper curve (Fauré-Fremiet & Dragiou, 1923)
△ rat (Stafford and Weatherall, personal communication)
▲ rabbit (Szendi, 1936; Shelley, unpublished)
▲ guinea-pig (Shelley, unpublished)

less of their relative maturity, cardiac glycogen varies inversely with maturity (fig. 3). Thus, although it is probable that, like the guinea-pig, sheep, rhesus monkey and man, all species have a foetal cardiac glycogen level of 35–40 mg./g. early in gestation, this falls during the latter part of gestation, and the level at birth varies enormously from species to species. The infant guinea-pig, born with its eyes open, plentifully covered with hair and able to run about and lead a relatively independent existence soon after birth, is born with near-adult cardiac glycogen levels of less than 5 mg./g.; whereas in the blind, naked, relatively helpless new-born rat the level is more than 25 mg./g. The rabbit is less mature at birth than the guinea-pig but more mature than the rat, and has a cardiac glycogen level of 5–14 mg./g. (Dawes, Mott & Shelley, 1959), similar to that in new-born kittens (see fig. 3). However, this is not a rigid relationship; although the lamb, pig and rhesus monkey are more mature at birth than the rabbit and cat, they also have cardiac glycogen levels of 10–20 mg./g. near term. The fall in cardiac glycogen appears to be associated with a particular stage in development which occurs in utero in the guinea-pig, mainly after birth in the rat, and partly in utero and partly after birth in the other species.

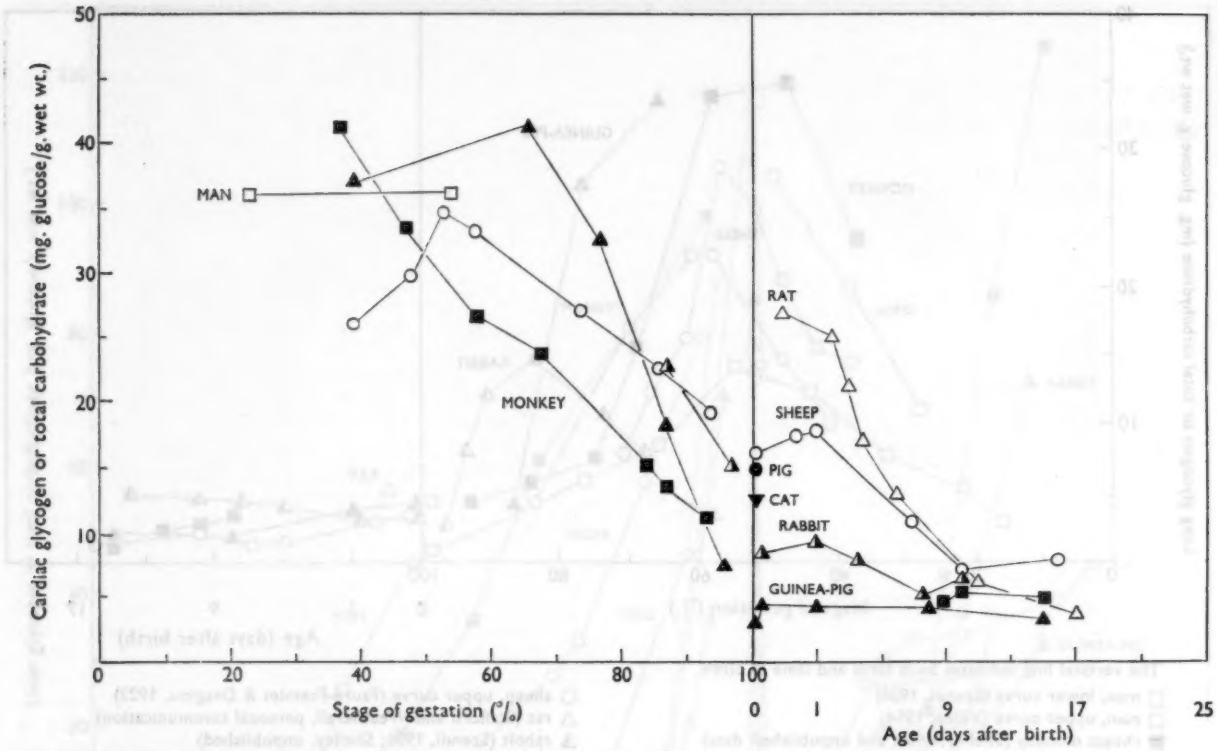
d. Skeletal Muscle

The amount of glycogen in skeletal muscle present at birth is also related to maturity (fig. 4), and it is tempting to suggest that species which are born capable of vigorous muscular activity and able to maintain their body-temperature need larger amounts of muscle glycogen than those born in a less mature condition. Thus, in the latter part of gestation, skeletal muscle glycogen rises to 30–40 mg./g. (about four times the adult level) in lambs and rhesus monkeys, to up to 25 mg./g. in guinea-pigs and to 15–18 mg./g. in dogs and rabbits; whereas new-born piglets have more than 70 mg./g., new-born rats have less than 13 mg./g. (Stafford & Weatherall, personal communication). Nevertheless, in all these species the muscle glycogen concentration at birth is usually at least twice the corresponding adult level and falls to or below the adult level within 1–3 days of birth. The rate of fall appears to be related to the activity of the new-born animal, since it is rapid in the pig (McCance & Widdowson, personal communication), lamb and guinea-pig, and probably in the kitten (Bernard, 1859b), slower in the rabbit and slowest in the rat.

e. Other Tissues

The only other foetal tissues for which there is quantitative information are the brain and kidney cortices, the skeleton and the placenta. The adult brain is notoriously poor in glycogen, and the foetal brain is no better off; Villee (1954) observed a slight fall in foetal brain glycogen, from 0.9 mg./g. to less than 0.2 mg./g., in man during the first half of gestation, and Shelley (1960) reported total carbohydrate levels of 0.5–1.6 mg./g. in lambs in the last two-thirds of gestation. Similar low values were observed in the brains of new-born

FIG. 3. Cardiac Glycogen in Different Species Before and After Birth



The vertical line indicates both term and time of birth.

- □ man (Villee, 1954)
- ■ rhesus monkey (Shelley, 1960, and unpublished data)
- ○ sheep (Shelley, 1960)
- ● pig (McCance & Widdowson, 1959)
- ▼ cat (Shelley, unpublished)
- △ rat (Dawes, Mott & Shelley, 1959)
- ▲ rabbit (Dawes *et al.* 1959; Shelley, unpublished)
- ▲ guinea-pig (Dawes *et al.* 1959; Shelley, unpublished)

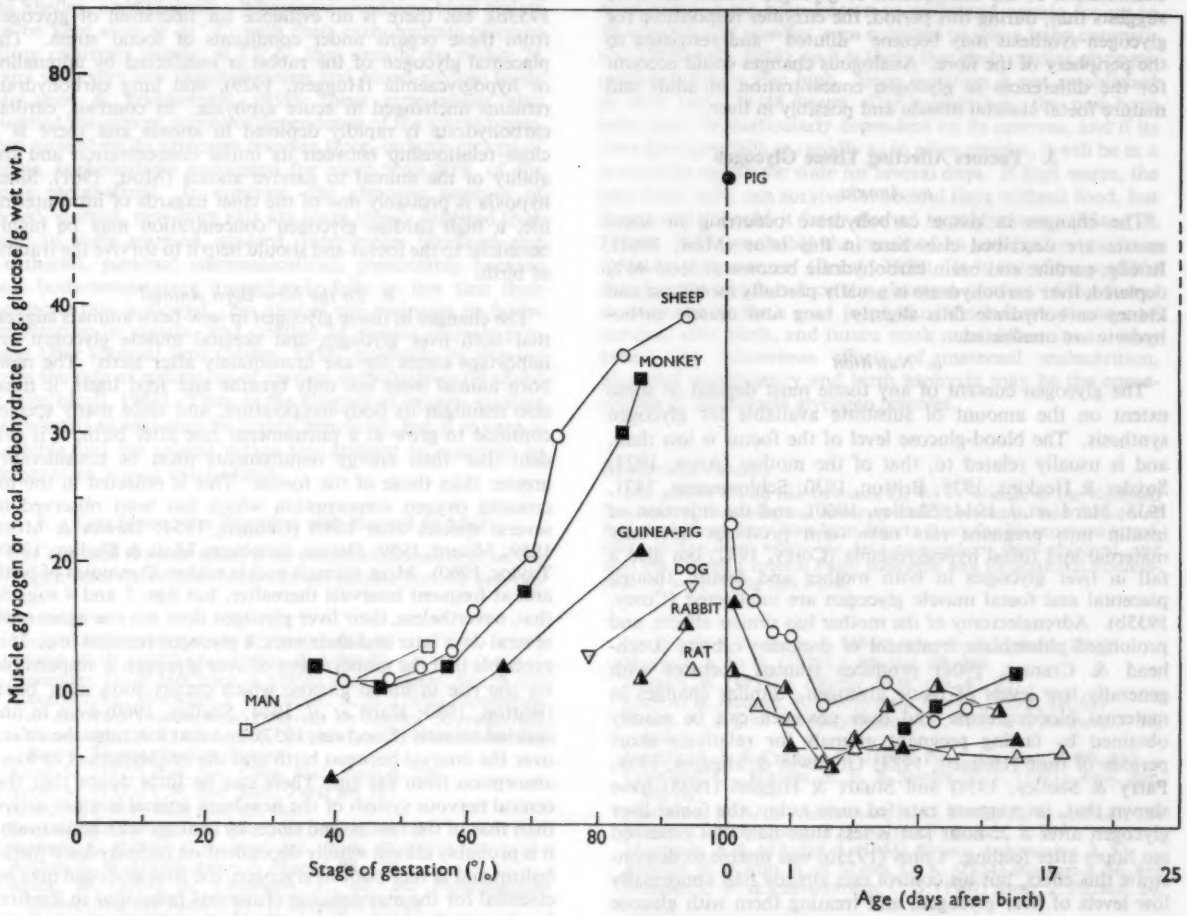
puppies, kittens and rats (Chesler & Himwich, 1943; Ferris & Himwich, 1946; Stafford, personal communication). Whereas in the new-born puppy and kitten the glycogen content of the cerebral cortex and caudate nucleus is less than that of the cerebellum, medulla and spinal cord, the reverse is true in adults, since there is a rise in forebrain glycogen and a fall in hindbrain glycogen with increasing age. The foetal kidney cortex contains rather more glycogen—2–3 mg./g. in man (Villee, 1953a)—and has a total carbohydrate content of 3–6 mg./g. in foetal lambs (Shelley, 1960), guinea-pigs and new-born rabbits (Shelley, unpublished); it is noteworthy that this is more than twice the adult level, and that the kidneys of new-born guinea-pigs contain less than 3 mg./g. Histological examination of foetal lambs' kidneys showed that the glycogen is concentrated in the tubular epithelium. The only foetal skeleton to be examined quantitatively is that of the pig, where Mendel & Leavenworth (1907) reported levels of up to 5 mg./g. in the last third of gestation.

The placenta of the rabbit is particularly rich in glycogen and contains peak amounts of up to 36 mg./g. at the 21st day of pregnancy (Huggett, 1929); most of this glycogen is in the maternal side of the placenta which may contain up to 56 mg./g., whereas the foetal side contains less than 6 mg./g. (Lochhead & Cramer, 1908; Loveland, Maurer & Snyder, 1931; Hoet & Brasseur, 1954). The glycogen content of the rabbit placenta decreases rapidly in the last third of gestation. The placentas of other species are less rich in glycogen, but in man (Szendi, 1934), the rat (Corey, 1935a) and the rhesus monkey (Shelley, 1960), glycogen does fall in the latter part of gestation. The guinea-pig placenta (Hard *et al.* 1944) and the placentas of ruminants (Bernard, 1859a) are poor in glycogen throughout pregnancy, but glycogen-rich "plaques" on the amnion and umbilical cord of ruminants disappear in the latter part of pregnancy.

2. Factors Responsible for Glycogen Formation

Little is known about the factors responsible for the first appearance of glycogen in foetal tissues and the subsequent changes in its concentration. Although there is a correlation between the appearance of islets of Langerhans in the foetal pancreas and the increase in foetal liver glycogen (Aron, 1922, 1931), and although experiments with decapitated foetuses suggest that this increase is dependent on foetal pituitary and adrenal function (Jacquot, 1955; Jost & Jacquot, 1955), the appearance of insulin and adrenocorticoids in the

FIG. 4. Skeletal Muscle Glycogen in Different Species Before and After Birth



The vertical line indicates both term and time of birth.
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○ sheep (Shelley, 1960)
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▽ dog (Schlossmann, 1938)
△ rat (Martínek & Mikuláš, 1954; Stafford and Weatherall, personal communication)
▲ rabbit (Shelley, unpublished)
▲ guinea-pig (Shelley, unpublished)

foetal circulation at this late stage in gestation would not explain the much earlier deposition of glycogen in the heart, lungs and skeletal muscle. Although a naturally headless, full-term rabbit foetus with normal litter-mates was devoid of liver glycogen, its skeletal muscle glycogen was normal (Shelley, unpublished), suggesting that foetal pituitary activity may not be essential for muscle glycogen formation. Liver glycogen increases rapidly in chickens shortly before hatching (Gill, 1938), but younger chicks injected with insulin do not accumulate liver glycogen, nor do immature chick embryos and immature liver cells which have been grafted on to the allantois of older chicks, even when a communal circulation has been established and the host's liver contains large amounts of glycogen (Guelin-Schedrina, 1936; Dalton, 1937). Nemeth, Insull & Flexner (1954) showed that liver slices from foetal guinea-pigs could not synthesize glycogen in vitro unless the foetus was of an age to synthesize liver glycogen in vivo; they concluded that the necessary enzyme system was incomplete until late in gestation and that the "branching enzyme" necessary for the synthesis of "glycogen primer" was among the last to appear.

It is therefore probable that the appearance of glycogen in a given tissue coincides with the development in that tissue of the enzymes necessary for glycogen synthesis and, since glycogen is a relatively non-diffusible substance, it is possible that changes in enzyme activity may account, at least partly, for some of the later changes in glycogen concentration. Whereas the decrease in glycogen concentration in the lungs is associated with the loss of a specific, glycogen-rich tissue, that in the heart is associated with a period of rapid growth during

which the cardiac muscle fibre increases in size; histological examination of the distribution of glycogen within the fibre suggests that, during this period, the enzymes responsible for glycogen synthesis may become "diluted" and restricted to the periphery of the fibre. Analogous changes could account for the differences in glycogen concentration in adult and mature foetal skeletal muscle and possibly in liver.

3. Factors Affecting Tissue Glycogen

a. Anoxia

The changes in tissue carbohydrate occurring in acute anoxia are described elsewhere in this issue (Mott, 1961). Briefly, cardiac and brain carbohydrate become at least 80% depleted, liver carbohydrate is usually partially mobilized and kidney carbohydrate falls slightly; lung and muscle carbohydrate are unaffected.

b. Nutrition

The glycogen content of any tissue must depend to some extent on the amount of substrate available for glycogen synthesis. The blood-glucose level of the foetus is less than, and is usually related to, that of the mother (Aron, 1923; Snyder & Hoskins, 1928; Britton, 1930; Schlossmann, 1931, 1938; Hard *et al.* 1944; Shelley, 1960), and the injection of insulin into pregnant rats near term produces not only maternal and foetal hypoglycaemia (Corey, 1932) but also a fall in liver glycogen in both mother and foetus, though placental and foetal muscle glycogen are unaffected (Corey, 1935b). Adrenalectomy of the mother has similar effects, and prolonged phlorrhizin treatment of pregnant rabbits (Lochhead & Cramer, 1908) produces stunted foetuses with generally low levels of tissue glycogen. Similar changes in maternal blood glucose and liver glycogen can be readily obtained by fasting pregnant animals for relatively short periods of time (Huggett, 1929; Goodwin & Higgins, 1934; Parry & Shelley, 1958) and Stuart & Higgins (1935) have shown that, in pregnant rats fed once a day, the foetal liver glycogen after a 22-hour fast is less than half that observed ten hours after feeding. Corey (1935b) was unable to demonstrate this effect, but his control rats already had abnormally low levels of liver glycogen, and treating them with glucose produced a rise not only in foetal and maternal liver glycogen but also in foetal muscle glycogen. Further work is needed to determine to what extent foetal tissue glycogen is affected by maternal nutrition, but preliminary experiments on pregnant guinea-pigs (Shelley, unpublished) suggest that as little as 48 hours of partial food restriction can reduce the cardiac carbohydrate of immature foetuses by nearly 40%, and that longer periods may have even more drastic effects.

4. Importance of Tissue Glycogen

a. To the Foetus

It is usually assumed that in the latter part of gestation foetal liver glycogen has functions similar to adult liver glycogen, and this hypothesis is supported by the fall in liver glycogen and rise in blood glucose observed in anoxia and by its depletion when the foetus is hypoglycaemic. Goldwater & Stetten (1947) estimated the normal rate of turnover of liver glycogen in the foetal rat to be as high as 5 mg./g./hr. Since large amounts of glycogen are present in the placenta and lungs at a time when there is very little in the liver, it has been suggested that these act as temporary glycogen stores until the liver is able to take over (Bernard, 1859a; Lochhead & Cramer, 1908; Szendi, 1934, 1936; Corey, 1935a; Villee, 1953b), but there is no evidence for liberation of glycogen from these organs under conditions of foetal stress. The placental glycogen of the rabbit is unaffected by adrenaline or hypoglycaemia (Huggett, 1929), and lung carbohydrate remains unchanged in acute asphyxia. In contrast, cardiac carbohydrate is rapidly depleted in anoxia and there is a close relationship between its initial concentration and the ability of the animal to survive anoxia (Mott, 1961). Since hypoxia is probably one of the chief hazards of intra-uterine life, a high cardiac glycogen concentration may be highly beneficial to the foetus and should help it to survive the trauma of birth.

b. To the New-Born Animal

The changes in tissue glycogen in new-born animals suggest that both liver glycogen and skeletal muscle glycogen are important stores for use immediately after birth. The new-born animal must not only breathe and feed itself, it must also maintain its body-temperature, and since many species continue to grow at a phenomenal rate after birth, it is evident that their energy requirements must be considerably greater than those of the foetus. This is reflected in the increasing oxygen consumption which has been observed in several species after birth (Gelineo, 1954; Dawes & Mott, 1959; Mount, 1959; Dawes, Jacobson, Mott & Shelley, 1960; Taylor, 1960). Most animals suckle within 30 minutes of birth and at frequent intervals thereafter, but figs. 1 and 4 suggest that, nevertheless, their liver glycogen does not rise again until several days later and their muscle glycogen remains low. It is probable that the mobilization of liver glycogen is responsible for the rise in blood glucose which occurs soon after birth (Britton, 1930; Hard *et al.* 1944; Shelley, 1960) even in unsuckled animals (Goodwin, 1957), and that this tides the infant over the interval between birth and the establishment of food absorption from the gut. There can be little doubt that the central nervous system of the new-born animal is more active than that of the foetus, and since, by analogy with adult brain, it is probably almost wholly dependent on carbohydrate metabolism and is very poor in glycogen, the liver glycogen may be essential for the maintenance of normal behaviour in the first few hours after birth. It is probable that the muscle glycogen can be utilized only by the muscle itself, though any lactate passing into the blood may be oxidized elsewhere. Foetal lambs which have been asphyxiated at birth and then resuscitated (Dawes, Mott, Shelley and Stafford, unpublished) sometimes lie on their sides for several hours making vigorous galloping movements, and when in extremis are devoid of liver glycogen and usually have blood-glucose levels below 1 mg./100 ml.; in such lambs the leg muscle carbohydrate is considerably depleted, but that in the back muscles is still high and that in the diaphragm has fallen to normal levels.

It is probable that muscle and liver glycogen are also essential for the maintenance of body-temperature. It is suggestive that, whereas new-born lambs and pigs can maintain their body-temperatures in a cool environment (Alexander & McCance, 1958; Mount, 1959) and have large muscle glycogen reserves (particularly the smaller, relatively naked, piglet), new-born rats with their small muscle glycogen reserves are almost poikilothermous (Adolph, 1951, 1957) and can only maintain their body-temperatures in the nest in association with their litter-mates. Lambs from ewes kept on a low plane of nutrition are less well able to maintain their body-tempera-

ture at birth (Alexander, McCance & Watson, 1956) and probably have less muscle glycogen than normal lambs (Parry and Shelley, unpublished). Whereas starved new-born piglets can be kept alive at 31° C. with their carbohydrate reserves falling relatively slowly, at 12° C. they usually die within 24 hours with very low tissue-glycogen and blood-glucose levels (McCance & Widdowson, 1959); these experiments also illustrate the almost complete dependence of the starved new-born animal on its glycogen reserves since, in both groups of piglets, carbohydrate accounted for more than 80% of the solids metabolized. In contrast, the glycogen reserves of cooled, starved, new-born rats are more slowly depleted than those of their warmed, starved, litter-mates (Stafford and Weatherall, personal communication), presumably because their body-temperature immediately falls so low that their metabolic rate is slowed. No animal can maintain its body-temperature in anoxia, and cooling of anoxic animals prolongs their survival time (Miller, 1958), probably by slowing down the rate of utilization of cardiac carbohydrate (Stafford & Weatherall, 1960). Thus, in the absence of oxygen, cooling a new-born animal may be highly beneficial, but if oxygen is available and the animal makes any attempt to maintain its temperature, it may well be fatal.

5. Conclusion: the Situation with Regard to Man

For obvious reasons there is no reliable information on the glycogen reserves of new-born human infants but, by analogy with other species, they probably have a high liver glycogen concentration and a fair amount of muscle glycogen. New-born babies have been known to withstand anoxia for up to 30 minutes and one has even survived to win a baby competition (Bullough, 1958), so it is likely that their cardiac glycogen concentration is also high. Since lactation is not established in man until 24–48 hours after parturition, the new-born baby must be particularly dependent on its reserves, and if its liver glycogen falls as rapidly as in other species, it will be in a precarious metabolic state for several days. If kept warm, the new-born baby can survive for several days without food, but if exposed to cold it later dies of hypoglycaemia (Mann & Elliott, 1957), probably the result of an attempt to maintain its body-temperature (Brück, 1959). It is therefore possible that factors which reduce the glycogen content of the foetus in utero or at birth may jeopardize the infant's chances of survival after birth, and future work must determine to what extent the deleterious effects of maternal malnutrition, placental insufficiency and birth asphyxia may be the consequence of inadequate glycogen reserves.

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THE ABILITY OF YOUNG MAMMALS TO WITHSTAND TOTAL OXYGEN LACK

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1 Temperature
2 Age, and maturity at birth
3 The importance of glycolysis
4 Carbohydrate reserves
5 Other factors
6 Conclusion
References

"... it appears, that those Animals continued 3 times longer in the Exhausted Receiver, than other Animals of that bigness would probably have done."

Thus Boyle (1670), using one-day "kitlings" in his pneumatical experiments, demonstrated the tenacity of life characteristic of the new-born. Elucidation of the reasons for this tenacity has been attempted both by studying the biochemical potentialities of foetal, as compared with adult, tissues, and by analysis of changes in the whole animal during anoxia.

We also have to consider the definition of survival. In the most extreme sense this means continuance of life without permanent impairment of any function; in practice it is necessary to select some arbitrary end-point for survival, such as the last gasp or the descent of the systolic pressure to a level at which revival is not possible.

1. Temperature

Bert (1870) concluded, from his experiments on the time taken for kittens to drown in water at different temperatures, ". . . il conviendra d'indiquer avec soin la température à laquelle on a opéré". The large surface area:weight ratio of new-born animals, coupled with poor insulation, favours heat loss, and the metabolic response to cold is readily suppressed by anoxia (for references see Hill, 1959 and Taylor, 1960). Metabolic rate falls as the animal cools, and survival time is therefore greatly increased at low temperatures (fig. 1a).

2. Age, and Maturity at Birth

From approximately mid gestation until some time in the early neonatal period, the younger the animal of any given species, the longer it survives total anoxia, whatever index of survival is taken as an end-point. The duration of survival of various isolated organ systems (e.g. spinal reflexes) of animals during anoxia also decreases steadily with increasing age (Enzmann & Pincus, 1934; Selle, 1941; Selle & Witten, 1941; Thoms & Hiestand, 1947). Though "maturity" does not easily lend itself to simple quantitative formulation (Tilney & Kubie, 1931), fig. 1b shows that the new-born of species which are relatively "mature" at birth (guinea-pig, sheep, monkey) survive anoxia less well than the obviously immature species. The consistency between the data of figs. 1a and 1b, derived from a variety of sources, is remarkably good.

3. The Importance of Glycolysis

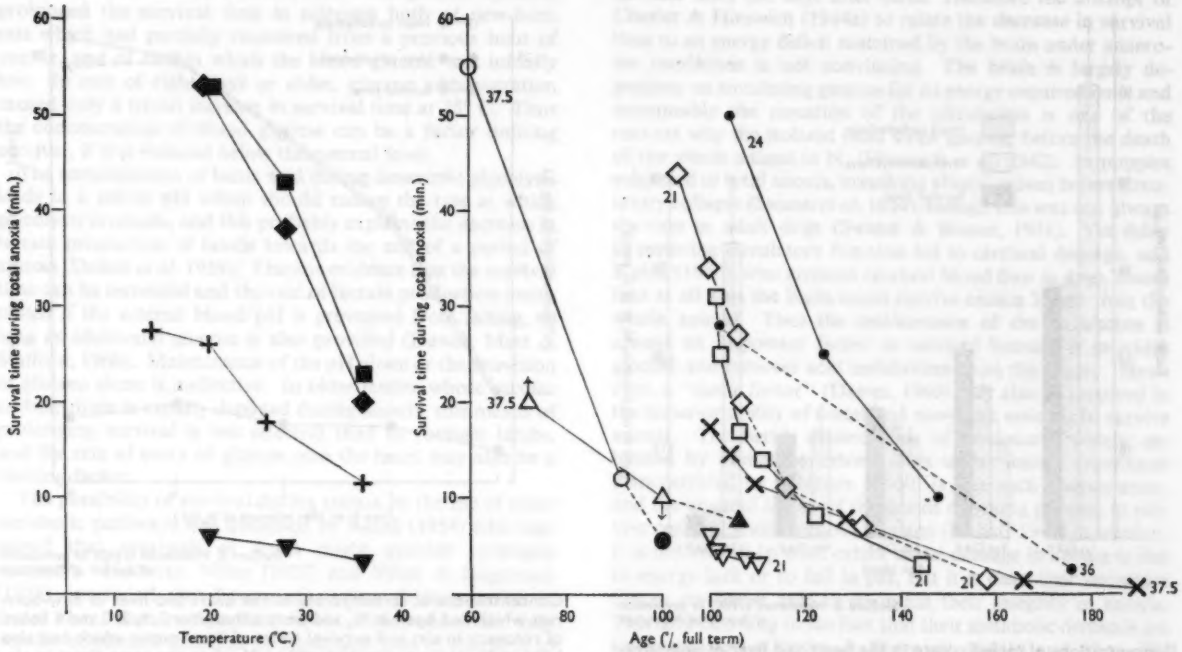
During total anoxia, some vital activities such as the circulation and breathing continue long after any oxygen remains in the blood (Swann, Christian & Hamilton, 1954; Dawes, Mott & Shelley, 1959; Dawes, Jacobson, Mott & Shelley, 1960), and it is evident that energy for these activities must be obtained by anaerobic processes.

Reiss (1931) observed that young rats and mice in the terminal stages of anoxia contained a higher concentration of lactic acid than did anoxic adults. The production of large amounts of lactic acid by young animals during anoxia was confirmed by Himwich, Bernstein, Herrlich, Chesler & Fazekas (1942). They also showed that inhibition of glycolysis by iodoacetate or sodium fluoride reduced the survival times of new-born rats in 100% N_2 from 50 minutes to 3 and 16 minutes respectively. It might therefore be supposed that young tissues are able to glycolyse faster, and indeed Villee (1957) found a higher rate of glycolysis in 8th-day than in 13th-day rat embryos at pH 6.9 in vitro; similarly Whittam (1960) reported that kidney slices from new-born rats and rabbits (which are born in an immature state) glycolysed faster than those from new-born guinea-pigs (which are born in a mature condition). Yet the maximum rate of glycolysis of many tissues increases with age (Chesler & Himwich, 1944a, 1944b; Villee, 1953, 1954; DeHaan & Field, 1959), and adult sheep breathing nitrogen accumulate lactic acid at least as fast as asphyxiated foetuses (Dawes *et al.* 1959). It would therefore appear that other factors may determine survival time during anoxia.

4. Carbohydrate Reserves

Born, Dawes & Mott (1955) showed that the foetal lamb, which weighs about 500 g. at 90 days' gestational age (full term is 147 days) cannot expand its lungs. In further experiments (Dawes *et al.* 1959) several blood samples were withdrawn during the course of anoxia lasting up to an hour, initiated by tying the umbilical cord. The blood pressure remained above 10 mm. Hg for 45 minutes on an average, the blood lactate increased rapidly and the blood pH fell precipitously to less than 7.0 within 10 minutes; samples of liver, lung and muscle showed no significant change in carbohydrate content. There was a small fall in kidney carbohydrate, and brain carbohydrate, which was initially very low, was almost completely depleted. Cardiac carbohydrate sustained a very large fall, from about 32 mg./g. to 5 mg./g. in 60 minutes. In foetal lambs of 121 days' gestation age or more, which were made anoxic and prevented from breathing, the initial level of carbohydrate in the liver was seven times that in younger lambs, and the blood sugar rose during anoxia. In spite of their higher blood sugar, the blood pressure of these older lambs had fallen to 10 mm. Hg and their cardiac carbohydrate was reduced from 17 mg./g. to 3 mg./g., after only 15 minutes. Thus, maintenance of the blood pressure above 10 mm. Hg was correlated with a cardiac carbohydrate level above 3–5 mg./g.; this point was reached more slowly by the younger lambs because they had more cardiac carbohydrate available at the beginning of anoxia and it was depleted less rapidly.

FIG. 1. Survival Time in Relation to Temperature and Age



1a. Time to Death of Anoxic New-Born Animals at Different Temperatures

Animals placed in 95% N_2 + 5% CO_2 (Miller, 1958):

- ▼ guinea-pigs
- ■ puppies
- ◆ rabbits

Animals killed by drowning (Bert, 1870):

- \+ kittens

1b. Survival Time During Total Anoxia in Relation to Age

Numerals show the temperature of the animal for sheep and monkeys, and of the environment for others.
The end-point is the time to last breath, except for foetal sheep (when it is the time at which arterial blood pressure reaches < 10 mm. Hg) and mice (when it is the time of extinction of spinal reflexes).

Anaesthetized foetus, cord tied:

- ○ sheep (Dawes, Mott & Shelley, 1959)
- ● sheep (Dawes, Mott & Stafford, 1960)
- △ monkey (Dawes, Jacobson, Mott & Shelley, 1960)

Animals placed in 100% N_2:

- ▲ monkey, anaesthetized (Dawes *et al.* 1960)
- ▽ guinea-pigs (Glass, Snyder & Webster, 1944)
- ◇—◇ rabbits (Caesarian) (Glass *et al.* 1944)
- ◇---◇ rabbits (born naturally) (Glass *et al.* 1944)
- □ puppies (Glass, Snyder & Webster, 1944)
- ●—● rats (Fazekas, Alexander & Himwich, 1941)
- ●---● rats (Stafford & Weatherall, 1960)

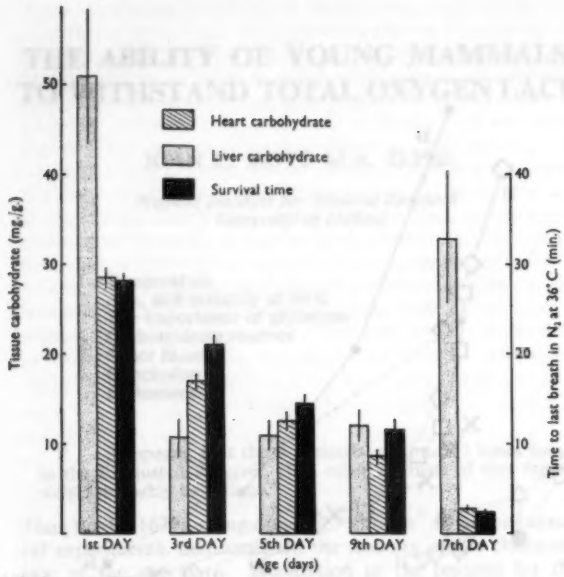
Animals killed by decapitation:

- × mice (Enzmann & Pincus, 1934)

More lactic acid accumulated in the heart than elsewhere, and calculation showed that the total lactate production was largely accounted for by the loss of carbohydrate from the heart. Stafford & Weatherall (1960) found that in rats 0–17 days old breathing 100% N_2, the only tissue, apart from the liver, showing large changes of carbohydrate content during anoxia was the heart, which was almost depleted when breathing stopped. During the first day of life the liver carbohydrate fell rapidly, but this had little effect on survival time which at all ages was very closely related to the initial cardiac carbohydrate concentration (fig. 2). When the carbohydrate stores were reduced by fasting or by anoxia the reduction in survival time was in direct proportion to the drop in initial cardiac carbohydrate (fig. 3). Thus the contention of Britton & Kline (1945) that the survival time of young rats during anoxia depends on the liver glycogen is untenable.

More details of the carbohydrate content of unasphyxiated foetal and new-born animals will be found in another paper (Shelley, 1961), which shows that the younger any given species of animal, the greater the concentration of carbohydrate in the heart. This is lowest at birth in the most mature

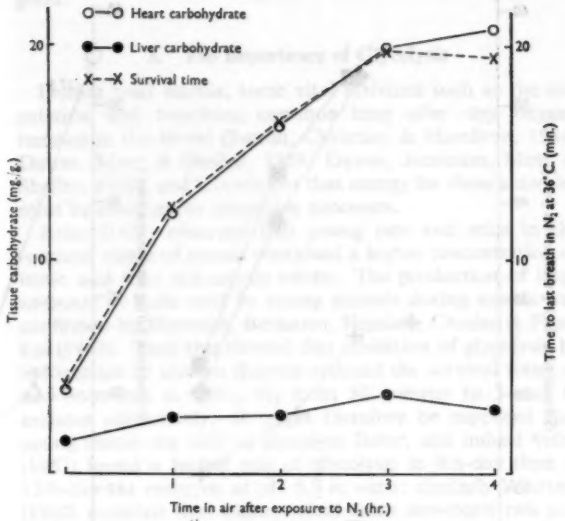
FIG. 2. Tissue Carbohydrate and Survival During Anoxia



Stafford & Weatherall (1960) by permission of Journal of Physiology

Concentrations of carbohydrate in the heart and liver of unasphyxiated new-born rats, and the survival times in N_2 of their littermates, during 17 days after birth.
The height of each block shows the mean of the 10 observations, and the vertical line represents the S.E. of that mean.

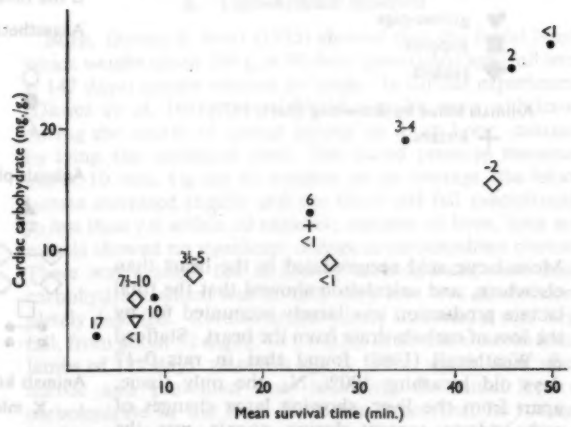
FIG. 3. Cardiac Carbohydrate and Survival During Anoxia


Heart carbohydrate
Liver carbohydrate
Survival time
Tissue carbohydrate (mg./g.)
Time to last breath in N_2 at 36° C. (min.)
Time in air after exposure to N_2 (hr.)

Stafford & Weatherall (1960) by permission of Journal of Physiology

Concentrations of carbohydrate in the heart and liver of new-born rats which had been in N_2 and were killed after 0, 1, 2, 3 and 4 hours of recovery in air; and survival times of litter-mates which had also been in N_2 and were re-exposed to N_2 after the same times in air. Each point is the mean of 5–6 observations.

FIG. 4. Cardiac Carbohydrate of Different Species and Survival During Anoxia



- ● rats at 24° C. (Fazekas, Alexander & Himwich, 1941)
- \+ kittens at 24° C. (Fazekas *et al.* 1941)
- ▽ guinea-pigs at 24° C. (Fazekas *et al.* 1941)
- ◇ rabbits at 21° C. (Glass *et al.* 1944)

The ages of the animals (in days) in relation to full term are shown by the figures next to each point.
The carbohydrate determinations are from Dawes, Mott & Shelley (1959) and Shelley (1961).

species (guinea-pig, 5 mg./g.), and highest in the least mature species (rat, 25 mg./g.), and fig. 4 shows that there is a close correlation between the cardiac carbohydrate concentration and the survival time during anoxia.

Glycolysis liberates only a minor proportion of the energy available in the glucose molecule, insufficient to maintain the body-temperature. At low temperatures, cardiac carbohydrate is glycolysed in vivo less rapidly (Stafford & Weatherall, 1960), and this accounts for the propensity of the anoxic animal to cool and to survive longer when cold. For this reason, care was taken to maintain artificially the temperature of the foetal lambs and of the new-born rats used in the experiments described above.

5. Other Factors

In 1942, Himwich *et al.* found that injection of insulin reduced the survival time to the last gasp of young rats breathing 100% N_2. Stafford & Weatherall (1960) confirmed this observation and showed that, although insulin greatly reduced the blood sugar, it caused only a small fall of cardiac carbohydrate and had an insignificant effect on the time to the last heart beat during anoxia. These experiments with insulin illustrate the difficulty which the selection of any single criterion of survival may lead to, in the interpretation of results. The survival time to the last gasp during anoxia in insulin-treated rats was restored to normal by the administra-

tion of glucose (Himwich, Fazekas & Homburger, 1943), and Stafford & Weatherall (1960) also found that glucose injection prolonged the survival time in nitrogen both of new-born rats which had partially recovered from a previous bout of anoxia, and of rats in which the blood glucose was initially low. In rats of eight days or older, glucose administration caused only a trivial increase in survival time at 35° C. Thus the concentration of blood glucose can be a factor limiting survival, if it is reduced below the normal level.

The accumulation of lactic acid during anaerobic glycolysis leads to a fall of pH which should reduce the rate at which glycolysis proceeds, and this probably explains the decrease in lactate production of lambs towards the end of a period of anoxia (Dawes *et al.* 1959). There is evidence that the survival time can be increased and the rate of lactate production maintained if the arterial blood pH is prevented from falling, so long as additional glucose is also provided (Dawes, Mott & Stafford, 1960). Maintenance of the pH alone or the provision of glucose alone is ineffective. In older lambs, whose cardiac carbohydrate is rapidly depleted during anoxia, this means of prolonging survival is less effective than in younger lambs, and the rate of entry of glucose into the heart may also be a limiting factor.

The possibility of survival during anoxia by the use of other metabolic pathways was discussed by Räihä (1954) who suggested that synthesis of lipids might provide hydrogen acceptors. However, Villee (1957) and Villee & Hagerman (1958) concluded that lipogenesis, although very active in foetal liver, does not proceed faster under anaerobic than aerobic conditions and that no significant gain of energy is to be expected from this source.

6. Conclusion

Bert (1870) considered and refuted the argument that the greater ability of the new-born to survive anoxia was due to the persistent patency of foetal circulatory channels, since, in the rat, for example, these are closed long before the survival time has fallen to adult levels.

The notion that the nervous system is the limiting factor in survival during anoxia has long been held, and Fazekas, Alexander & Himwich (1941) were impressed by the fact that the oxygen uptake of rat brain tissue in vitro increases with age, while the survival time of the whole rat in N_2 decreases during the first fortnight of life. But in fact their evidence shows that survival time begins to decrease within 24 hours of birth, while the oxygen uptake of the brain in vitro does not increase until ten days after birth. Therefore the attempt of Chesler & Himwich (1944a) to relate the decrease in survival time to an energy deficit sustained by the brain under anaerobic conditions is not convincing. The brain is largely dependent on circulating glucose for its energy requirements, and presumably the cessation of the circulation is one of the reasons why the isolated head stops gasping before the death of the whole animal in N_2 (Himwich *et al.* 1942). In puppies subjected to total anoxia, breathing always ceased before circulatory collapse (Swann *et al.* 1954), though this was not always the case in adult dogs (Swann & Brucer, 1951). Yet delay in restoring circulatory function led to cerebral damage, and Kabat (1940), who arrested cerebral blood flow in dogs, found that at all ages the brain could survive anoxia longer than the whole animal. Thus the maintenance of the circulation is always an important factor in survival because it provides glucose and removes acid metabolites from the tissues. However, a "tissue factor" (Dawes, 1960) may also be involved in the superior ability of foetal and new-born animals to survive anoxia. The better maintenance of potassium content exhibited by immature kidney slices under anoxic conditions demonstrated by Whittam (1960) is one such phenomenon, and the unusual ability of the brains of young puppies to survive cerebral stasis without damage (Kabat, 1940) is another. It is not known to what extent tissue damage in anoxia is due to energy lack or to fall in pH, but it is clear that immature tissues are better able to maintain their integrity in anoxia. This may be owing to the fact that their metabolic demands are sometimes less (Chesler & Himwich, 1944b) and, if so, the available substrate will last longer and the pH fall more slowly.

The practical conclusion to be drawn from the present state of knowledge of the conditions under which anoxic survival is possible, is that the carbohydrate stores, particularly of the heart, should be protected from dissipation. Re-synthesis of cardiac carbohydrate does occur (see fig. 3) but may be limited by the rate of entry of glucose into the cell. Thus the factor of vital importance is the store of carbohydrate already available within the cardiac cells. Repeated episodes of anoxia or hypoxia are cumulatively as well as individually dangerous and it is probable that, if the pH is not restored to normal levels, a stage is reached at which glycolysis may be completely inhibited. Repeated anoxic episodes may also exhaust the liver glycogen and lead to hypoglycaemia, so that maintenance of the circulation might be insufficient to protect the brain from injury.

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CHANGES IN THE CIRCULATION AT BIRTH

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1 The circulation in the mature foetus
2 The onset of breathing after birth
3 Closure of the ductus arteriosus
4 Closure of the foramen ovale
5 Changes in cardiac output after birth
6 Conclusion
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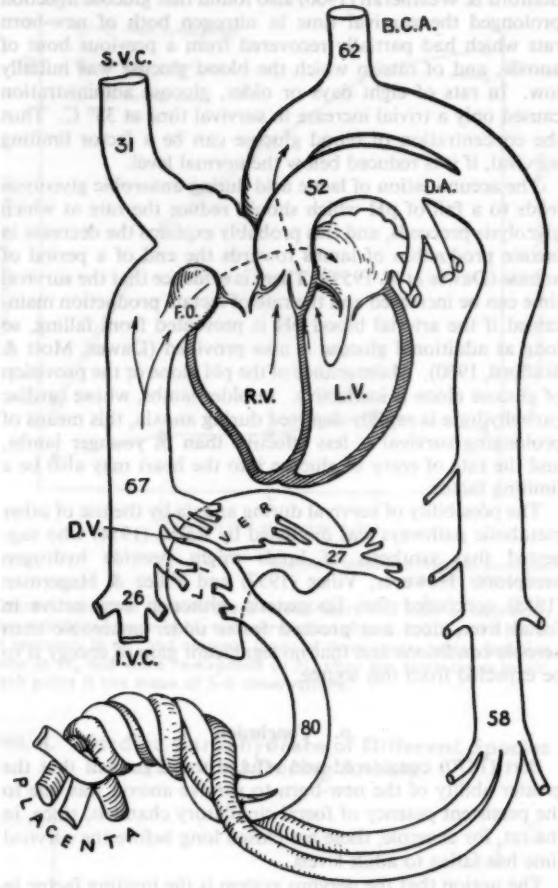
1. The Circulation in the Mature Foetus

The first direct experiments on the circulation and respiration of foetal mammals were performed by Huggett in 1927. He showed that in the foetal goat the carotid arterial blood contained more oxygen than did umbilical arterial blood. This demonstrated that the various streams of blood, one from the placenta which is fairly well oxygenated, and others from the foetal tissues which contain much less oxygen (see fig. 1), do not mix completely in the great veins or in their passage through the heart. Observations by Barcroft (1946) in the foetal lamb confirmed this conclusion, but he found that the difference in the O_2 content of blood from the two arteries was variable, possibly because the blood samples were not withdrawn simultaneously. It seemed desirable to obtain more information, which might lead to a quantitative analysis of the distribution of blood flow. Observations were made on lambs (Dawes, Mott, Widdicombe & Wyatt, 1953; Dawes, Mott & Widdicombe, 1954), and on rhesus monkeys (Dawes, Jacobson, Mott & Shelley, 1960), as shown in fig. 2. The agreement between the observations on the two species was good, and it was interesting that the difference between the O_2 content of blood from the ascending and descending aorta did not appear to vary with the absolute level of the O_2 saturation.

Barcroft and his colleagues also considered the reasons why the blood streams entering the heart did not mix thoroughly. They pointed out (Barclay, Franklin & Prichard, 1944; Barcroft, 1946) that the foramen ovale lay between the inferior

FIG. 1. The Foetal Circulation in the Lamb



Born, Dawes, Mott & Widdicombe (1954) by permission of Cold Spring Harbor Symposia on Quantitative Biology

I.V.C.: inferior vena cava
S.V.C.: superior vena cava
R.V.: right ventricle
L.V.: left ventricle
D.V.: ductus venosus
F.O.: foramen ovale
D.A.: ductus arteriosus
B.C.A.: brachiocephalic artery

The figures indicate the mean O_2% saturation of blood withdrawn simultaneously and averaged from estimations on six lambs.

ABILITY OF YOUNG MAMMALS TO WITHSTAND TOTAL OXYGEN LACK

Continued from page 147

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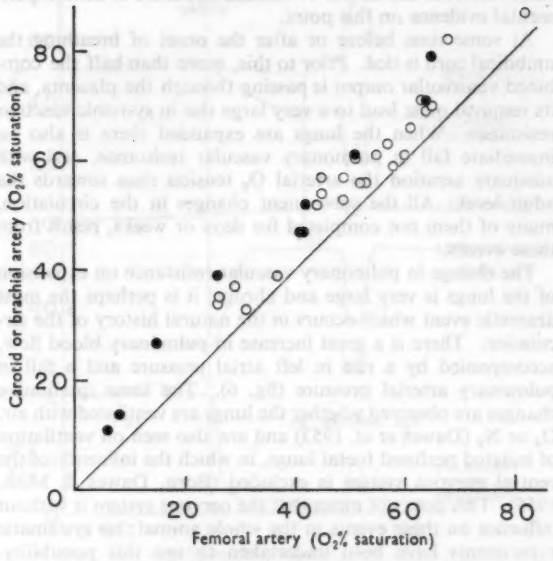
FIG. 2. O_2 Saturation of Carotid and Femoral Blood

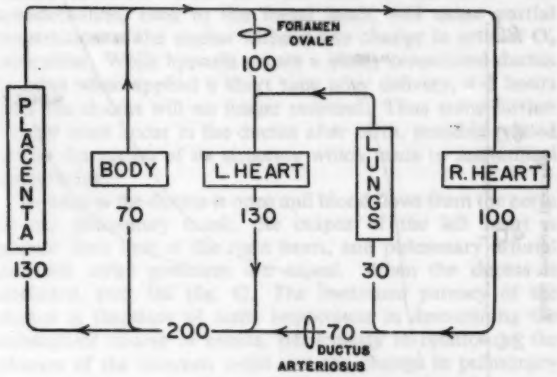


Blood samples were withdrawn simultaneously from branches of the ascending aorta (brachial or carotid artery) and descending aorta (femoral artery).

vena cava and the left atrium, not between the two atria. Cine-angiograms on foetal lambs showed that, whereas contrast medium injected into the umbilical vein or the inferior vena cava passed at once and simultaneously into both atria, contrast medium injected into tributaries of the superior vena cava entered the right atrium only (none was seen during the next few seconds in the left atrium or ventricle, the ascending aorta or its branches). Similarly, injections of radio-iodinated serum albumin ^{131}I into the external jugular veins of foetal lambs led to the appearance of no radioactive tracer in the carotid artery for 10–12 seconds thereafter, as compared with the appearance of large amounts of tracer within 2–3 seconds of an inferior vena caval injection (Dawes, Groom, Mott, Rowlands and Thomas, unpublished). Therefore we can be tolerably sure that the course of the circulation in the great veins of the foetus really does correspond with that shown in fig. 1. The cine-angiographic observations of Lind & Wegelius (1954) suggest that this is also true of the human foetus.

The next problem was to provide some indication of the probable volumes of blood flow within the foetal circulation. These vary under different physiological conditions, but it is useful to have a model from which to start. At various points in the circulation two streams of different O_2 content mix. Thus, in the right atrium, blood containing 67% O_2 from the inferior vena cava mixes with blood containing 31% O_2 from the superior vena cava and coronary sinus (fig. 1). The O_2 content of pulmonary arterial blood was 52%, and from this it was possible to calculate the relative contribution from each of the great veins. By an extension of this method it proved possible to calculate the relative volume of blood flow through

FIG. 3. Schematic View of the Foetal Circulation



L. heart: left heart R. heart: right heart

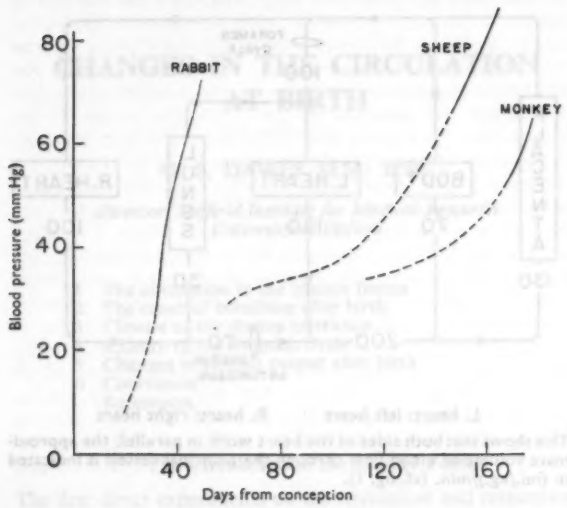
This shows that both sides of the heart work in parallel; the approximate volume of blood flow through the principal vessels is indicated in (ml./kg.)/min. (cf. fig. 1).

each of the main vessels (Dawes *et al.* 1954). Figure 3 shows that, of the combined output of both ventricles, the placenta takes over 50%, the forequarters and hindquarters ("body") together about 30%, and the lungs less than 15%. Thus the placenta is a relatively low-resistance circuit and the lungs a high-resistance circuit, both in parallel with the foetal tissues.

These calculations can be checked directly. Umbilical blood flow has been measured by a foetal plethysmograph (Cooper, Greenfield & Huggett, 1949; Acheson, Dawes & Mott, 1957) or, more reliably, by inserting a flowmeter into the umbilical vein within the abdomen (Dawes, Mott & Rennick, 1956; Dawes & Mott, 1959b). The volume of umbilical blood flow in mature lambs was 100–180 (ml./kg.)/min. Total pulmonary blood flow has not been measured, but flow through the left lung is 5–10 (ml./kg.)/min., which is reasonable. It is obviously desirable to obtain other direct measurements of blood flow in the foetus, particularly of right and left heart output. Since the two ventricles work in parallel it is not necessary that their outputs should be identical.

Adequate nervous control of the circulation and respiration is of obvious importance at birth. Yet in the past there have been suggestions that autonomic nervous control in the new-born animal is deficient (Kellogg, 1927; Clark, 1935; Bauer, 1939). If this were so it would be expected to be deficient, or even absent, in the foetus. The blood pressure rises during gestation and after birth (fig. 4), but this does not necessarily mean that the cardiovascular reflexes are developed after birth. Yet there is evidence that in the foetal lamb administration of cyanide induces respiratory movements at 60–70 days' gestation (term is 147 days) (Barcroft & Karvonen, 1948); that the carotid sinus depressor reflex is active at term (Donatelli, 1940; Ingiulla, 1940; Cross & Malcolm, 1952); and that many other autonomic nervous pathways are functional during the last third of gestation (Born, Dawes & Mott, 1956). Even the new-born rabbit, which appears much less mature than the lamb (in that it is blind, naked and can barely crawl), possesses a full complement of circulatory and respiratory reflexes. Thus stimulation of the peripheral end of the vagus causes profound bradycardia (Dawes, Handler & Mott,

FIG. 4. Blood Pressures Before and After Birth



Broken line: before birth
Continuous line: after birth

Figure shows the rise of blood pressure with increasing age, before and after birth, in the three species investigated.

1957). The Hering-Breuer and carotid body chemoreceptor reflexes and the pulmonary respiratory chemoreceptor reflex (Dawes & Mott, 1959a), as well as the baroreceptor reflexes (Downing, 1960), are all active. Although the blood pressure of a new-born rabbit is less than 40 mm. Hg, yet a rise of pressure in one vascularly isolated carotid sinus to as little as 20 mm. Hg causes a fall in systemic arterial blood pressure (fig. 5). Similarly in the new-born monkey, occlusion of the carotid arteries causes a rise of arterial pressure which is abolished by cutting the carotid sinus nerves (Dawes *et al.* 1960).

2. The Onset of Breathing after Birth

Figures 1 and 3 show that the arterial blood supply to the foetal tissues (apart from the lungs) is derived from branches of the ascending and descending aorta. The mean arterial O_2 saturation in lambs under barbiturate anaesthesia was 62% (ascending aorta) and 58% (descending aorta), as shown in fig. 1, and in rhesus monkeys 67% and 58% respectively (Dawes *et al.* 1960). These probably represent optimal conditions. The O_2 saturation in the umbilical arteries of human infants and rhesus monkeys after delivery is on the average less than 30%. The fall in arterial O_2 content which takes place during delivery is probably due to compression of the umbilical cord, partial separation of the placenta and also to a reduction in uterine blood flow during contractions. Thus in rhesus monkeys in utero there is a small but significant fall in arterial O_2 saturation during strong uterine contractions, even though the cord and placenta are intact (Dawes *et al.* 1960).

In the foetal lamb a reduction in arterial O_2 saturation below 50% is associated with a fall in O_2 consumption and a rise in blood lactate (Dawes & Mott, 1959b). These are unequivocal signs of anoxia, and we have to look no further for an adequate stimulus to breathing, since the chemoreceptor reflexes are active at birth. It is probable that sensory stimuli to the body, and particularly the change in external temperature, reinforce the effects of anoxia, but there is little experimental evidence on this point.

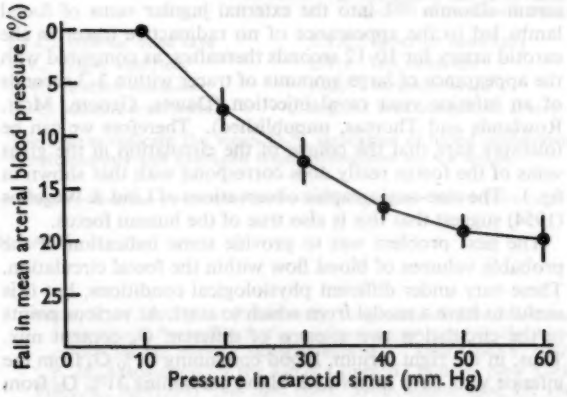
At some time before or after the onset of breathing the umbilical cord is tied. Prior to this, more than half the combined ventricular output is passing through the placenta, and its removal must lead to a very large rise in systemic vascular resistance. When the lungs are expanded there is also an immediate fall in pulmonary vascular resistance, and with adequate aeration the arterial O_2 tension rises towards the adult level. All the subsequent changes in the circulation, many of them not completed for days or weeks, result from these events.

The change in pulmonary vascular resistance on expansion of the lungs is very large and abrupt; it is perhaps the most dramatic event which occurs in the natural history of the circulation. There is a great increase in pulmonary blood flow, accompanied by a rise in left atrial pressure and a fall in pulmonary arterial pressure (fig. 6). The same qualitative changes are observed whether the lungs are ventilated with air, O_2 or N_2 (Dawes *et al.* 1953) and are also seen on ventilation of isolated perfused foetal lungs, in which the influence of the central nervous system is excluded (Born, Dawes & Mott, 1955). This does not mean that the nervous system is without influence on these events in the whole animal; no systematic experiments have been undertaken to test this possibility. Nevertheless it is likely that the principal effect of gaseous expansion of the lungs on pulmonary vascular resistance is due to some simple mechanical action on the smaller blood vessels. It is a phenomenon which unquestionably deserves further study.

3. Closure of the Ductus Arteriosus

The ductus arteriosus does not close at once after birth. It constricts at first rapidly, so that its lumen is considerably narrowed within 10–30 minutes after adequate ventilation has

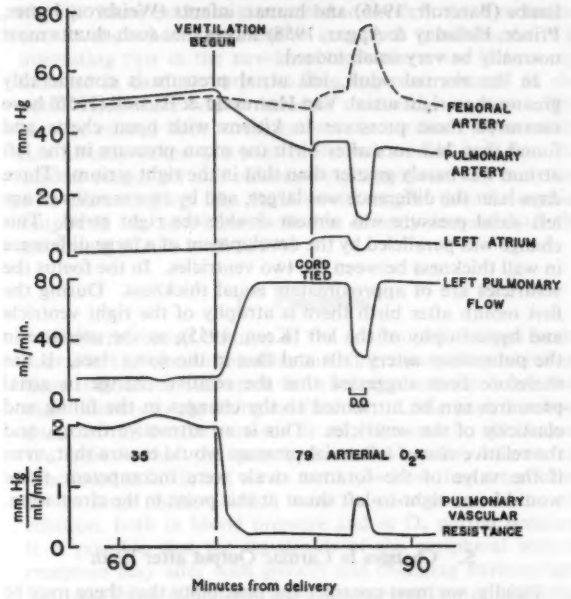
FIG. 5. The Baroreceptor Reflex in the New-Born Rabbit



Downing (1960) by permission of Journal of Physiology

Observations from nine new-born rabbits to show the fall in mean systemic blood pressure at different pressures applied to the vascularly isolated left carotid sinus.
The vertical lines indicate the standard error of the means.

FIG. 6. Changes in the Circulation on Ventilating the Foetal Lung



Modified from Dawes, Mott, Widdicombe & Wyatt (1953)

D.O.: ductus arteriosus occluded

Lamb (2.6 kg.) anaesthetized with dial-urethane. Artificial positive pressure ventilation of the lungs caused a large fall of pulmonary vascular resistance, an increase in pulmonary flow and a fall in pulmonary arterial pressure. Temporary occlusion of the ductus arteriosus caused a rise in femoral pressure and a fall in pulmonary pressure and flow, showing that blood had been flowing from the aorta into the pulmonary trunk. The figures 35 and 79 indicate the carotid arterial O_2% saturation.

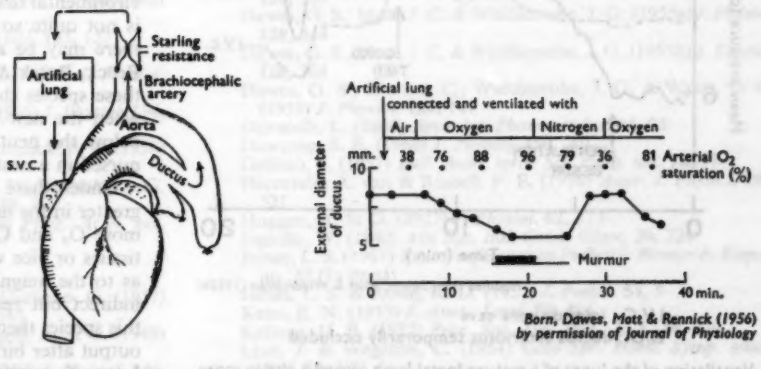
begun in the lamb. Because of the rise in systemic vascular resistance and the fall in pulmonary vascular resistance, the direction of blood flow through the ductus normally reverses in this and other species (e.g. calf and foal), so that a loud murmur is audible over the left side of the chest for a period of hours or days from birth (Dawes, Mott & Widdicombe, 1955b; Amoroso, Dawes & Mott, 1957). Thereafter it constricts more slowly, the murmur becomes fainter and disappears, and finally anatomical obliteration of the lumen takes place.

The mechanisms which are responsible for the initial constriction of the ductus have been analysed in the lamb. The changes in transmural pressure do not appear to be relevant, since the ductus will constrict when the arterial O_2 saturation is raised in an isolated heart–ductus–artificial lung preparation, as shown in fig. 7 (Born, Dawes, Mott & Rennick, 1956). This experiment suggests that the effect of an increase in arterial pO_2 in causing constriction of the ductus is probably due to a direct action on the very muscular wall of the vessel. However, in the intact animal there are undoubtedly other factors involved. Thus administration of adrenaline and noradrenaline, even to the foetal lamb, will cause partial constriction of the ductus without any change in arterial O_2 saturation. While hypoxia causes a partly constricted ductus to relax when applied a short time after delivery, 4–5 hours later the ductus will no longer respond. Thus some further change must occur in the ductus after birth, possibly related to the disruption of its structure which leads to anatomical obliteration.

So long as the ductus is open and blood flows from the aorta to the pulmonary trunk, the output of the left heart is greater than that of the right heart, and pulmonary arterial and left atrial pressures are raised. When the ductus is occluded, they fall (fig. 6). The continued patency of the ductus is therefore of some importance in determining the subsequent course of events, particularly in relation to the closure of the foramen ovale and the change in pulmonary arterial pressure after birth. Yet pulmonary arterial pressure does not immediately fall to adult levels, even when the ductus is occluded. It is possible that the progressive fall in pulmonary arterial pressure which takes place for some days or weeks after birth may be related to a change in calibre and structure of the vessels consequent on the fall in transmural pressure. Little account has so far been taken of any changes in cardiac output which may follow delivery, a point which will be considered later.

Finally, so long as the ductus arteriosus is patent, it is possible that blood may flow through it in the foetal direction, from pulmonary trunk to aorta. This indeed appears to be the normal state of affairs in the human infant for up to an hour after birth (James, 1961). Yet after this time both cineangiography and blood-oxygen determinations suggest that blood flow is in the opposite direction (Adams & Lind, 1957; James & Rowe, 1957), and a transient murmur attributed to flow through a constricted ductus has been described by Burnard (1958). It is certainly possible that the time course of the vascular changes may vary somewhat in different species.

FIG. 7. The Effect of Oxygen upon the Ductus Arteriosus



Born, Dawes, Mott & Rennick (1956) by permission of Journal of Physiology

S.V.C.: superior vena cava

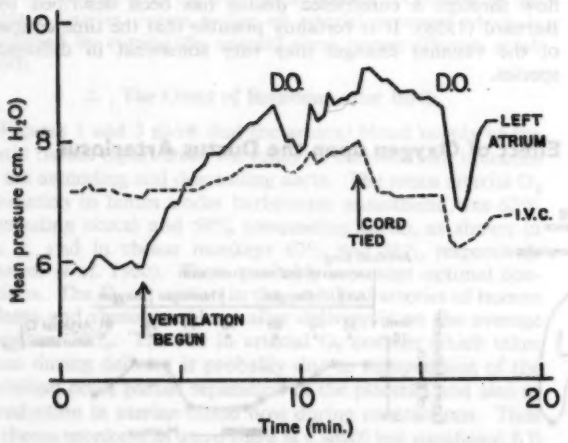
Heart–ductus arteriosus–artificial lung preparation from mature foetal lamb. Ventilation of the artificial lung with O_2 caused a rise in arterial O_2 saturation and constriction of the ductus arteriosus.

4. Closure of the Foramen Ovale

Measurement of the pressure difference across the foramen ovale in the lamb showed that this was reversed within a minute or two of full expansion of the lungs. In the foetus about half of the blood flowing up the inferior vena cava passes through the foramen ovale (fig. 3). The valve of the foramen ovale is thus held open during most of the cardiac cycle. When ventilation begins, left atrial pressure rises and soon exceeds inferior vena caval pressure (fig. 8). In addition, when the umbilical cord is then tied, there is a fall in inferior vena caval pressure, because the large venous return from the placenta has been interrupted (fig. 8). This further accentuates the pressure gradient across the foramen ovale. At this time the phasic pressure pulses in the inferior vena cava and left atrium, which before ventilation were similar in appearance, now become totally different (Dawes, Mott & Widdicombe, 1955a). However, part of the large pressure difference which has developed across the foramen ovale is then due to the patency of the ductus arteriosus, since occlusion of the ductus causes a greater fall in left atrial pressure than in inferior vena caval pressure (fig. 8).

Anatomical closure of the foramen ovale occurs at different time intervals after birth in different species. It occurs within a few hours in the foal. In the lamb it begins towards the end of the first week (Dawes *et al.* 1955a), but no sign of permanent closure was seen in rhesus monkeys 8–12 days old (Dawes *et al.* 1960). Until anatomical closure occurs, it is always possible that the foramen ovale may open at some part of the cardiac cycle, to form a right-to-left shunt. Recent observations by Condorelli, Dagianti, Polosa & Giuliano (1957), using a dye-dilution method involving the injection of 0.2 ml.,

FIG. 8. Closure of the Foramen Ovale



Modified from Dawes, Mott & Widdicombe (1955b)

I.V.C.: inferior vena cava
D.O.: ductus arteriosus temporarily occluded

Ventilation of the lungs of a mature foetal lamb caused a rise in mean left atrial pressure. When the umbilical cord was tied the I.V.C. pressure fell. Ventilation of the lungs, tying the cord and the continued patency of the ductus all contribute to the rapid reversal of the pressure gradient across the foramen ovale after birth.

suggest that a shunt may exist up to the fifth day from birth, in lambs. But the high arterial O_2 saturation in new-born lambs (Barcroft, 1946) and human infants (Weisbrot, James, Prince, Holaday & Apgar, 1958) shows that such shunts must normally be very small indeed.

In the normal adult, left atrial pressure is considerably greater than right atrial. Van Harreveld & Russell (1956) have measured these pressures in kittens with open chests and found that 24 hours after birth the mean pressure in the left atrium was barely greater than that in the right atrium. Three days later the difference was larger, and by two months of age left atrial pressure was almost double the right atrial. This change was paralleled by the development of a large difference in wall thickness between the two ventricles. In the foetus the ventricles are of approximately equal thickness. During the first month after birth there is atrophy of the right ventricle and hypertrophy of the left (Keen, 1955), as the pressure in the pulmonary artery falls and that in the aorta rises. It has therefore been suggested that the relative change in atrial pressures can be attributed to the changes in the filling and elasticity of the ventricles. This is an attractive theory, and the relative rise of left atrial pressure would ensure that, even if the valve of the foramen ovale were incompetent, there would be no right-to-left shunt at this point in the circulation.

5. Changes in Cardiac Output after Birth

Finally, we must consider the possibility that there may be large changes in cardiac output and in systemic blood flow as well as in pulmonary blood flow after birth. To put this matter in its proper perspective we have to consider the changes in O_2 consumption which occur after birth. Only in the lamb do we as yet have measurements of foetal O_2 consumption under stable physiological conditions, and in the absence of anoxia. These measurements show that there is no significant change in O_2 consumption per unit of body-weight during the last third of gestation, but there is almost a threefold increase in consumption within 24 hours of birth (Dawes & Mott, 1959b). In the monkey it increases not quite twofold in 8–10 days (Dawes *et al.* 1960). In the dog and pig it is probably intermediate (Gelineo, 1957; Mount, 1959). The rat shows a rather smaller increase over the first 2–3 days of life (Taylor, 1960). All these observations were made at the neutral environmental temperature[1]. The situation in the human infant is not quite so certain, though here too it is probable that there may be an increase (Cross, Tizard & Trythall, 1957; Brück, Brück & Lemtis, 1958). There is no doubt that in all these species the increase in O_2 consumption is considerable when the new-born animal is maintained at a temperature below the neutral, as is the case even when the young are nursed in a nest.

Under these circumstances the task of the circulation is greater in the new-born than in the foetus, since considerably more O_2 and CO_2 have to be shifted from the lungs to the tissues or vice versa. Only in the lamb is there any evidence as to the magnitude of the change. Calculation, admittedly indirect but reasonably well substantiated, suggests that in this species there may be a nearly threefold increase in cardiac output after birth (Cross, Dawes & Mott, 1959). Thus in the foetus the mean cardiac output has been estimated at about

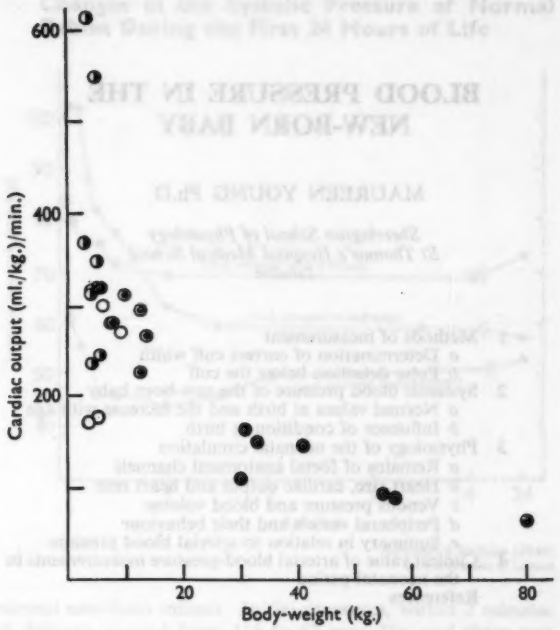
[1] See Hill, p. 164 of this number of the Bulletin.—Ed.

115 (ml./kg.)/min. (one ventricle only) as compared, in 16 new-born lambs, with a mean output of 325 ± 30 ml./kg./min. (fig. 9). More evidence is required to confirm this conclusion and to show how the increase in output is achieved. It is interesting that in the new-born lamb there was little or no increase in cardiac output during hypoxia, whereas in the adult sheep there was a threefold to fivefold increase. This may be related both to the increase in cardiac output which has already taken place at birth and to the fact that the cardiac output per unit of body-weight progressively decreases with increasing age (fig. 9). It would suggest that the cardiac output in the new-born lamb has already increased to the maximum (Cross *et al.* 1959).

6. Conclusion

While the broad outlines of the anatomical changes in the course of the circulation after birth are now well established, there is evidently still much to be learnt about the details and their relation to physiological function. Little work has been done on the ductus venosus or on the systemic circulation. We believe that the principal cardiovascular reflexes are functional at birth, but we do not know how they are affected by the large changes which occur at some points in the circulation, both in blood pressure and in O_2 partial pressure. It is possible that the thresholds of the peripheral sensory receptors may alter with growth and changing environment. We need more direct and more accurate estimates of cardiac output in the foetus and in the infant after birth. Finally, our knowledge has been derived from observations on rather few species and, of these, lambs have been used a great deal. In so far as information is available on the human infant, the general principles seem to apply, but more experiments on other species would be helpful.

FIG. 9. Changes in the Cardiac Output After Birth



Cross, Dawes & Mott (1959) by permission of Journal of Physiology

◑: 1-day-old lambs ○: 20–60-day-old lambs
○: 2–12-day-old lambs ●: adult sheep

The cardiac output/kg. body-wt. has been plotted against body-wt. Compare with right-heart output shown in fig. 3 for foetal lambs.

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BLOOD PRESSURE IN THE NEW-BORN BABY

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1. Methods of Measurement

The dimensions and lack of co-operation of the new-born babe have made great demands upon the ingenuity of investigators, and this is reflected in the numerous methods which have been described to make arterial blood-pressure measurements practicable. Rucker & Connell (1924), Abt & Feingold (1930), Londe (1932) and Salmi (1936) review the early literature. The conventional inflatable cuff and manometer are now generally used; the width of the cuff has been shown to be critical but there is still no agreement as to the best method of detecting the pulse below it.

a. Determination of Correct Cuff Width

The indirect method of arterial blood-pressure measurement was compared with the direct method, using a membrane manometer, in the new-born infant (Woodbury, Robinow & Hamilton, 1938) and small children (Robinow, Hamilton, Woodbury & Volpitto, 1939). In the infant, direct measurements, from the umbilical artery, were compared with those by the cuff method and palpation at the wrist: in the older infants and children the two methods were compared for the brachial artery, auscultation below the cuff being possible in the majority of cases. Robinson *et al.* (1939) wrote:

> The proper width of the cuff increases with the circumference and with the length of the arm and probably decreases with the compressibility of the arm. All three factors vary in such a manner that the proper width of the cuff increases with age.

It was also observed that the systolic and pulse pressures were increased in the brachial artery immediately above the occlusion, but that this was less pronounced in the youngest infants where the contour of the peripheral pulse wave did not differ greatly from the central contour; however, this was a factor which increased the proper cuff width for systolic pressure measurement.

The proper cuff width for diastolic pressure also increased with the age of the child; the correlations between direct and indirect methods were less good, probably owing to the difficulties encountered with auscultation in small arms, and the correct width for diastolic pressure measurement was a little lower than that required for systolic pressure. Robinow *et al.* (1939) decided that results for diastolic pressure with the indirect method would have meaning only in grossly abnormal cases; in subsequent years no advance has been made with indirect diastolic pressure measurement during the first few years of life.

Subsequent to these comparisons between direct and indirect methods of blood-pressure measurement, it has been customary to use a 2.5-cm. (1-inch) cuff for systolic pressure measurement in the new-born; it is important that it should be long enough, 8–10 cm., to encircle the upper arm. Before 1938 a cuff 4–6 cm. wide was generally used by paediatricians and falsely low readings were obtained. Robinow *et al.* (1939) found that a 5-cm. cuff gave correct readings for children of from six months to one year of age, and a 9-cm. cuff for those of 1–13 years.

b. Pulse Detection Below the Cuff

In new-born infants, Salmi (1936), Browne & Dodds (1936) and Schaffer (1955) were able to use auscultation for the measurement of systolic pressure, and Ashworth, Neligan & Rogers (1959) found it possible in a certain number, but tiring. In children over six weeks of age auscultation was quite practical for systolic pressure measurement (Robinow *et al.* 1939). Palpation of the radial artery has been used to determine systolic pressure in the new-born, but it has not been considered entirely satisfactory (Seitz & Becker, 1920; Reis & Chaloupka, 1923; Wolff, 1930; Woodbury *et al.* 1938; Brash, 1949). Holland & Young (1956) found palpation of the brachial artery at the elbow easier. Rucker & Connell (1924) observed the oscillations of the mercury manometer attached to the occluding cuff, and made estimates for both systolic and diastolic pressures, while both Balard (1912a) and Bowman (1933) used a Pachon oscillometer, the blood-pressure apparatus used on the mainland of Europe, in which the pulse is also recorded from the occluding cuff.

The objectivity and sensitivity of electrical and optical methods for indicating the pulse are most desirable features, but none of these methods has yet been compared with that of direct measurement, and all involve the use of apparatus which is not simple and compact enough for everyday clinical use. Schaffer (1955) used an impedance plethysmograph to record pulsations beyond the occlusion cuff, Keuth (1955) used a very sensitive sphygmograph, Rice & Posener (1959) a differential optical pulse indicator, and Morse, Brownell & Currens (1960) incorporated a crystal microphone in the occlusion cuff. Schaffer could obtain reproducible results only with a 4-cm. cuff.

Celander & Thunell (1960) use a venous occlusion plethysmograph, on the foot and leg, for measuring arterial pressures; systolic pressure is the highest pressure in the occluding cuff at which there is a minimal arterial inflow, and diastolic pressure the highest cuff pressure at which there is maximal flow. This is a research method providing valuable data on blood flow as well as on arterial pressure.

The difficulties of palpation have resulted in the recent

development of two methods more suitable for the clinician. The first is the "flush" method of Goldring & Wohltmann (1952) and Cappe & Pallin (1952). A cuff is placed on the forearm or leg, and the hand or foot is exsanguinated by wrapping tightly with rubber sheeting or crepe bandage. The cuff is inflated well above the expected systolic pressure and the bandage removed. The pressure in the cuff is slowly released and the reading observed at the first sign of a flush, when the blood re-enters the exsanguinated limb. When the limb is warm and has been blanched completely, and if the pressure is released at about 5 mm./sec., the end-point is quite distinct. The simplicity of the method has resulted in its wide-spread use (Reinhold & Pym, 1955; Sullivan & Kobayashi, 1955; Forfar & Kibel, 1956; Moss, Liebling, Austin & Adams, 1957); all agree that the reproducibility of the method is good, especially if a 4-cm. cuff is used, and its accuracy is sufficient to demonstrate pressure differences of clinical significance. Comparisons of the method with intra-arterial measurements show that it indicates mean arterial pressure; Moss *et al.* (1957) have also shown that the readings are independent of the width of the occlusion cuff. However, this method seems to me clumsy and disturbing to the infant.

The second method which eliminates the difficulties of palpation is due to Fordtran, Killinger & Newsom (1957) and to Ashworth *et al.* (1959). The latter use an inflatable cuff, 2.5 cm. in diameter, on the upper arm, and a second cuff on the forearm, which is attached to a pulse indicator consisting of a glass capillary containing an interrupted column of xylol; a wider, sealed tube beyond the capillary acts as an air cushion. The lower cuff is inflated to about 60 mm. Hg and the xylol beads respond to the transmitted arterial pulse. The upper cuff is inflated until the pulsations disappear; systolic pressure is taken as the reading when regular pulsations reappear in the xylol during deflation. The xylol beads will reach maximum excursion at the diastolic pressure; this is difficult to judge by eye and the readings are unreliable. The results for systolic pressure are easily reproducible and, in a small number of observations, good agreement has been obtained with the direct method (Neligan & Smith, 1960). The apparatus is inexpensive and simple, fitting into the usual sphygmomanometer box. Several readings a minute may be taken. This pulse indicator has the merit that systolic pressure is detected in the second cuff by a change from no pulsations to the appearance of pulsation, while in the old oscillometers a change in pulsation was observed only with the occlusion cuff (Evans & Mendelssohn, 1946).

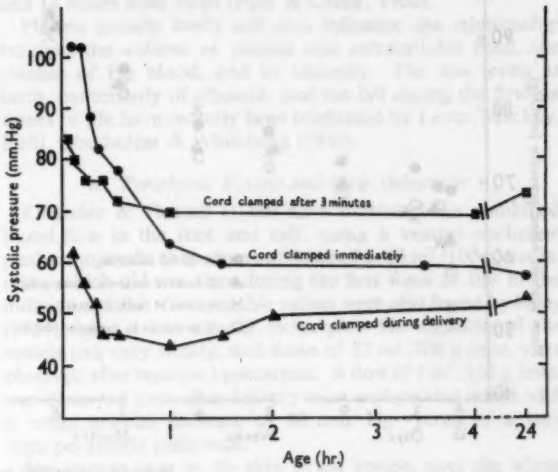
2. Systemic Blood Pressure of the New-Born Baby

a. Normal Values at Birth and the Increase with Age

The earliest value given for umbilical arterial pressure in the human infant is 64 mm. Hg (Ribemont, 1879). Seitz (1901) and Haselhorst (1929) observed figures of 73 mm. Hg and 76–110 mm. Hg respectively. Woodbury *et al.* (1938) recorded an average of 80/46 mm. Hg in 24 normal infants. These investigators found no change in blood pressure with the onset of respiration and when the cord is clamped, findings which have recently been confirmed by Wallgren, Karlberg & Lind (1960). This is in contrast with the observation made on the lamb (Dawes, 1958).

Ashworth & Neligan (1959) have reported marked changes in systolic pressure, within the first 24 hours of life, in 20

FIG. 1. Three Representative Records of the Changes in the Systolic Pressure of Normal Babies During the First 24 Hours of Life



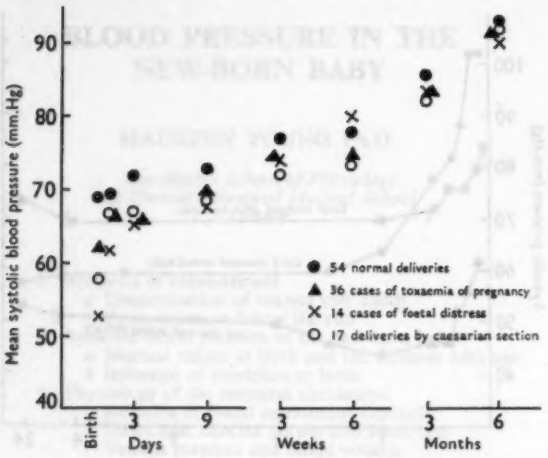
Ashworth & Neligan (1949) by permission of Lancet

normal new-born infants. Initial pressures, within 2 minutes of delivery, ranged from 116 to 62 mm. Hg, and there was subsequently a fall varying from 14 to 54 mm. Hg, to minimum pressures of 74–44 mm. Hg (for examples, see fig. 1). Delay in clamping the cord, to allow blood transfusion from the placenta (Gunther, 1957), postponed, but did not affect, the magnitude of this fall in pressure. The lowest pressure was observed within four hours of delivery if the cord was clamped during or immediately after birth, otherwise, within 24 hours. Clamping the cord caused a small but delayed rise of pressure; Woodbury *et al.* (1938) also observed a rise in mean pressure and a change in pulse contour only when the cord was occluded near the umbilicus.

During the second 24 hours of life Ashworth & Neligan (1959) observed a gradual rise in pressure of 17–28 mm. Hg, but the levels never exceeded those immediately after delivery; thereafter there was a steady rise in pressure during the first week. This increase in systolic pressure during the first days and week of life has been observed repeatedly and demonstrates the reproducibility, in the hands of a given observer, of the wide variety of methods used (Seitz & Becker, 1920; Reis & Chaloupka, 1923; Rucker & Connell, 1924; Londe, 1932; Bowman, 1933; Woodbury *et al.* 1938; Brash, 1949; Forfar & Kibel, 1956). The results of Holland & Young (1956), shown in fig. 2, are representative of the general pattern found. It is also seen that there is a slow rise in systolic pressure during the following nine months of life. Robinow *et al.* (1939) have shown, by intra-arterial measurement, that in the subsequent twelve years there is a very gradual rise in both systolic and diastolic pressures. Many more direct readings are needed before normal changes at birth and the rate of rise of the arterial blood pressure in the infant and young child can be clearly defined.

Woodbury *et al.* (1938) observed direct readings of 39/21 mm. Hg in a five-month foetus and 55/25 mm. Hg at six and a half months' gestation; in infants born at seven and eight

FIG. 2. Mean Systolic Blood Pressures at Birth and During the First Few Months of Life, in Normal Infants



Holland & Young (1956) by permission of British Medical Journal

months the arterial pressure was still below that of full-term infants. Reis & Chaloupka (1923) found a low pressure in premature infants, and Holland & Young (1956) found an average systolic pressure of 54 mm. Hg in 16 premature infants; all were under 2.5 kg. at birth, the blood-pressure range was large, 35–70 mm. Hg, and the values bore no relation to the maturity of the infant, though there was a possible relation to the birth-weight.

The relation between systolic pressures measured in the arm and leg is important to establish, since upon this depends the diagnosis of coarctation of the aorta (Sullivan & Kobayashi, 1955; Reinhold & Pym, 1955; Moss, Liebling & Adams, 1958; Forfar & Kibel, 1956). The results, so far, have all been obtained with the flush method; they are conflicting and further observations using other methods are required before the relationship between the two pressures is known.

Large increases in pressure were observed during the act of crying, by Woodbury *et al.* (1938) and Young & Holland (1958); smaller rises were recorded by Forfar & Kibel (1956).

b. Influence of Condition at Birth

Figure 2 shows that infants who have been suffering from foetal distress, and those delivered by Caesarian section, have a lower mean systolic pressure than follows uncomplicated deliveries; the blood-pressure level of these babies recovered to normal levels within a few days, and the subsequent rise was indistinguishable. The measurements after the normal deliveries were made within an hour of birth, and those after abnormal deliveries within 15 minutes; in view of the description by Ashworth & Neligan (1959) of the early changes in pressure, more precise timing should have been made in order that these early readings could be comparable both within the group and between groups. The blood pressure was not significantly lower after breech and forceps deliveries, nor after the administration of pethidine (meperidine hydrochloride) and other analgesics to the mother before delivery (Bowman, 1933; Holland & Young, 1956). Reis & Chaloupka (1923) did not consider that Caesarian section influenced neonatal blood pressure, but observed high readings after forceps delivery especially when "high" forceps were applied. Bowman also found higher pressures after cerebral trauma, which could be reduced by lumbar puncture.

Pre-eclamptic toxaemia of the mother, and its influence on the blood pressure of the new-born infant, have been of great interest on account of the possible placental transfer of a chemical substance, associated with the hypertension, from the maternal to foetal circulations. Woodbury *et al.* (1938) did find significantly higher neonatal pressures, and suggested that the toxaemic substance was rapidly destroyed after birth, because the subsequent levels were comparable with those in babies born of normal mothers. No elevation of the neonatal blood pressure was found by Reis & Chaloupka (1923), Brash (1949) and Holland & Young (1956); the latter found that the first readings obtained were low, but concluded that this was owing to the inclusion of babies born by Caesarian section, which they had shown to lower the early neonatal values (fig. 2). It is also possible that the circulation in the new-born is not capable of responding to this hypothetical toxaemic substance.

3. Physiology of the Neonatal Circulation

The flow of blood through tissues depends upon the pressure gradient between the arteries and the smaller vessels; the arterial pressure is provided by the energy of the heart pump and is opposed by the resistance in the peripheral vessels. In order to find out what is happening in the circulation, simultaneous measurements of systemic arterial blood pressure and blood flow, at least, need to be made and the peripheral resistance calculated. It will be apparent in the succeeding paragraphs that most of the data have been obtained in isolation from other circulatory measurements. The mechanisms responsible for the gradual rise in arterial pressure throughout gestation are temporarily interrupted by parturition and, later, modified by the environment. The major circulatory readjustments at birth, demonstrated in animals by Barcroft (1946) and Dawes (1958), are common to the human infant (Lind & Wegelius, 1954), but the subsequent modifications are likely to be different for each species, depending upon orthostatic factors, mode of life, and so forth.

a. Remains of Foetal Anatomical Channels

Studies of dye-dilution curves, cardiac murmurs and the oxygen saturation of the blood in the heart and extremities have provided evidence for patency of both the foramen ovale and the ductus arteriosus, intermittently or continuously, for about a fortnight after birth in normal children. The literature is reviewed by Dawes (1961).

b. Heart Size, Cardiac Output and Heart Rate

On the day of birth the average heart volume, as determined radiologically, for 35 full-term infants was 48 ml. (Kjellberg, Rudhe & Zetterström, 1954); during the first hour of life there is a 20% increase in volume, with a return to the immediate postnatal level within three hours (Lind & Wegelius, 1954). During the subsequent four days the volume diminishes by 25% and thereafter increases; this decrease in heart size during the early days of life is more marked in the premature baby.

Cardiac output in new-born infants has been measured by the dye-dilution technique (Prec & Cassels, 1955) and from oxygen consumption and arteriovenous-oxygen differences in cardiac blood (Adams & Lind, 1957). The range observed was wide, 180–850 ml./min. (probably accounted for by variable shunts), but the average, 540 ml./min., corresponded to a cardiac index of 2.5 l./min. and agreed with theoretical calculations based on the surface area of the infant; assuming a heart rate of about 140/min., the stroke volume is therefore approximately 4 ml. Prec & Cassels (1955) made all their observations within 2–26 hours of birth and found the lowest values in the youngest babies; when a left-to-right shunt was present the cardiac output reached 2 l./min.

The extreme lability of the infant heart rate has resulted in a few published records of the changes after birth. Young & Holland (1958) and Ashworth & Neligan (1959) made careful studies on small numbers of infants and show that, in spite of the wide range, there is a fall during the first 48 hours of life, as was observed by Balard (1912b); this was followed by a steady rise during the subsequent week.

c. Venous Pressure and Blood Volume

The pressure of blood as measured in a vein is the resultant of two opposing forces, the positive pressure transmitted from the arteries and the negative pressure exerted upon the column of blood from the thoracic cavity; the former dominates in the peripheral veins, but as its value diminishes centrally the influence of the negative intrathoracic pressure becomes important. The effective pressure determining the venous blood flow is the total gradient from the pressure measured in the periphery to the pressure measured in the right auricle; the latter is approximately equal to the intrapleural pressure and normally fluctuates during the respiratory cycle between 3 mm. and 6 mm. Hg below atmospheric pressure, and is taken arbitrarily as the central reference pressure. Therefore, the site of any venous pressure measurement must be clearly stated and accompanied by a simultaneous reference pressure, that most easily obtained being the intra-oesophageal pressure. Venous pressure measurements in infants have all been made with reference to the sternal angle (Mollison & Cutbush, 1949; Bonham Carter, Bound & Smellie, 1956; Taylor, Birchard, Bright & Egan, 1958). No one has made sure that the sternal angle is the correct reference pressure for the new-born child, and it may well be different in the baby with respiratory distress. During the first 24 hours of life the venous pressure, measured through the umbilical vein, is +3 to +5 cm. H_2O, in the supine position.

Mollison, Veall & Cutbush (1950) made a careful study of the blood volume in infants, between the ages of 1½–6 hours and 6–24 hours, finding the average 85 ml./kg. with a range of 68–115 ml./kg. Similar values were found by Sisson & Whalen (1960), who have demonstrated, by the dye technique, the changes in circulating blood volume which occur within the first six hours of life, in normal infants whose cords were tied immediately: during this period there is a 22.5% increase in blood volume, consisting of a 25.6% and 20% rise in red-cell and plasma volumes respectively. This extra blood has probably been trapped in some of the peripheral vessels cut off from the circulating blood by the high arteriolar tone developed during birth asphyxia, and may also be expelled from the blood reservoirs during the change from the foetal to adult circulatory pattern. From a study of blood haemoglobin and plasma composition, Gairdner, Marks, Roscoe & Brettell (1958) came to quite the opposite conclusion: namely, that there was haemoconcentration during the first hours of life. Extracellular fluid volumes are not known during this time but have been shown to remain unchanged between 6 and 16 hours after birth (Fink & Cheek, 1960).

Plasma protein levels will also influence the relationship between the volume of plasma and extracellular fluid, the volume of the blood, and its viscosity. The low levels at birth, particularly of albumin, and the fall during the first six weeks of life have recently been confirmed by Levin, Mackay, Neill, Oberholzer & Whitehead (1959).

d. Peripheral Vessels and their Behaviour

Celander & Thunell (1960) have measured the combined blood flow in the foot and calf, using a venous occlusion plethysmograph; they observed flows of 5–10 ml./100 g. tissue/min., which did not alter during the first week of life in the individual child. Comparable values were also found by Riley (1954) using a less elegant technique. The capacity of the vessels can vary widely, and flows of 27 ml./100 g./min. were observed after reactive hyperaemia. A flow of 1 ml./100 g./min. was observed soon after delivery in an asphyxiated infant with a mean arterial pressure of 80 mm. Hg—proof of a very high peripheral resistance.

Vasomotor tone in the skin blood vessels, over the whole body, has been demonstrated by Brück, Brück & Lemtis (1957) and in the upper and lower limbs by Bower (1954) and Young (unpublished). The indirect heating test of Lewis & Pickering (1931) was used and, as both the latent period of the rise in skin temperature and the rate of rise after the commencement of body warming are comparable with those of the adult, the activity of the vasomotor sympathetic mechanisms appears to be well developed. Since there is dilatation in both the upper and lower extremities, there are both release of sympathetic constrictor tone and activation of sympathetic vasodilator fibres (Roddie, Shepherd & Whelan, 1957). During the first three days of life, skin dilatation is not so readily observed, and this is possibly related to the lower body-temperatures and to the relative asphyxia of birth.

The response of the systolic blood pressure and of the pulse rate to tilting into the "feet down position" was studied during the first 12 days of life in order to determine whether the baroreceptors were active at this time (Young & Holland, 1958). There was no change in the systolic pressure on tilting during the first four days, and thereafter there was a small rise, suggesting that the baroreceptors were active; but before any conclusions are drawn the experiments need repeating with intra-arterial pressure measurements. It is possible that the rise in pressure and cardiac acceleration were the responses of an aroused infant, and evidence of increased excretion of sympathetic amines under such circumstances has been obtained (Greenberg, Lind & von Euler, 1960).

The influence of moderate doses of acetylcholine, adrenaline and noradrenaline on the pulmonary circulation has been observed after the injection of these drugs into the right heart in infants under 36 hours of age (Adams, Lind & Rauramo, 1958); the systolic pressure rose following adrenaline and noradrenaline, but there was no hypotension following acetylcholine. Adrenaline caused tachycardia; noradrenaline, bradycardia; and acetylcholine, no change in heart rate. In older children, James & Rowe (1957) found a rise in pulmonary arterial pressure during the breathing of mixtures low in oxygen, and a marked fall in systemic pressure

when the arterial oxygen was reduced to 55%. Adams *et al.* (1958) observed no consistent response of the pulmonary arterial pressure to hypoxia, but there was always a tachycardia. It is difficult to assess the sensitivity of the cardiovascular system to chemical stimuli before more information is available as to the individual responses of the heart, the blood vessels and the activity of the regulatory mechanisms which will influence the final response; the effect of patent foetal channels will also have to be taken into account.

The chemical environment of the blood vessels of the new-born differs from that of the adult, and will probably influence their tone and their responses. Plasma sodium and calcium are essentially the same as in the adult, but the potassium is high and the pH and anion concentration vary widely (Smith, 1959; James, 1959). Even less is known of the hormonal environment of the blood vessels in the neonatal period than in the adult. West, Shepherd, Hunter & MacGregor (1953) have shown that the amine composition of the supra-renal and accessory organs changes during the first year of life in the human infant; noradrenaline is excreted in larger quantities than adrenaline in the new-born infant (Greenberg *et al.* 1960). Norris and Stacey (unpublished) find low 5-hydroxytryptamine concentrations in the platelets of umbilical vein blood, suggesting another difference in the hormonal environment.

e. Summary in Relation to Arterial Blood Pressure

The systolic pressure, measured within two minutes of delivery, varies widely, and this may be fairly attributed to at least two unknown factors: the duration of asphyxia and the disturbance of body-fluid distribution by the pressures exerted upon the baby during parturition. The fall in pressure during the early hours of life may partly be explained by the decrease in the peripheral resistance as the vasomotor centre and blood vessels recover from the effects of asphyxia. Celander (1960) has shown that the resistance is high in distressed infants, and may take several days to fall.

After the immediate readjustments of birth the mean arterial pressure of the new-born infant, at rest, is 30 mm. Hg below the mean pressure of the young adult. The cardiac output of the infant/kg. body-wt. is about double that of the adult, and the blood flow to the extremities is likewise double, so that the lower arterial pressure may be accounted for by a low peripheral resistance. This low resistance would appear to be due to low tonic activity of the mechanisms, probably both chemical and nervous, concerned with the regulation of blood flow through vessels other than those supplying the skin; the physiological activity of the vasomotor-sympathetic mechanism to the skin vessels in the infant has been shown to be comparable with that of the adult, and these vessels are not usually considered to take part in the redistribution concerned with the regulation of blood pressure. Development of the chemical and reflex tone of the peripheral vessels will contribute relatively more to the gradual rise in arterial pressure during the subsequent months and years of life than the cardiac output which declines in relation to the body-weight.

4. Clinical Value of Arterial Blood-Pressure Measurements in the Neonatal Period

At present, the clinical value of arterial blood-pressure measurement in the new-born and young infant has been in the diagnosis of coarctation of the aorta. When further information is available on the relationship between arterial pressure and the mode of delivery, on asphyxia at birth, and on haemorrhage and the quantity of blood transfused from the placenta, repeated observations using such a simple method as that of Ashworth *et al.* (1959) could be a useful guide to the condition of an infant—at birth and during the subsequent hours. It could also be a guide to the effectiveness of any treatment necessary.

The 4-cm. cuff is gaining in popularity but it must be remembered that, however reproducible the results may be, the available evidence indicates that the blood-pressure readings will be low.

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* * *

RESPIRATION IN THE NEW-BORN BABY

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1 Central and reflex gaseous control
a Acidosis
b Alkalosis
c Oxygen lack
2 Foetal respiratory reflexes
3 Lung inflation in the new-born
References

This paper will not be concerned with the mechanics of established breathing, nor with the special pressure–volume relationship associated with the first breaths taken in extra-uterine life. The pioneer work in this field of Karlberg and his colleagues has been reviewed recently (Karlberg, 1960).

However, attention must be drawn to the striking results of Avery & Mead (1959) who have studied the surface tension properties of saline extracts from the lungs of very premature foetuses, from infants with "hyaline membrane" disease and from normal lungs. The premature and hyaline lung extracts have surface tensions around 30 dynes/cm., while normal lung extracts have less than 10 dynes/cm. This work (which follows from that of Pattle, 1955) suggests the possibility that collapse of the lung may be due to the instability of an alveolus lined by a fluid with a high surface tension (that of plasma is about 55 dynes/cm.). The nature of this surface-tension-lowering material in mature lung is not known, and one awaits with interest identification of what must be a wetting agent of very ancient lineage.

This paper will be limited to a discussion of the central and reflex control of respiration so far as it has been studied in the new-born.

1. Central and Reflex Gaseous Control

It is well known that adult man, for each 100 ml. of oxygen taken up by the tissues, needs to ventilate his lungs with 2.2–2.5 litres of air. This "ventilation equivalent for oxygen" holds over a large range of individuals who are either at rest or exercising with moderate severity (with an O_2 consumption of 200–2,500 ml./min.). Thus Gray (1950) summarizes the data from 11 different authors who made 611 determinations of oxygen consumption and total ventilation in 86 subjects at differing work levels. The same relationship between volume of air breathed and O_2 consumed also exists between different resting individuals with varying metabolic rates. Boyer & Bailey (1943) found that the difference in basal metabolic rate between two selected groups was 50%, and that the difference in ventilation volume was 51%.

Certain circumstances will alter the ventilation equivalent for oxygen. Thus, respiratory or metabolic acidosis will tend to raise the ratio, as will excessive heating of the body. Respiratory or metabolic alkalosis, on the contrary, will lower the ratio. Figure 1 shows some values for this ratio in the premature and full-term new-born baby. Even over the very small range of volumes of pulmonary ventilation in different babies at rest (about threefold), there is a highly significant correlation between oxygen consumed and minute volume of ventilation ($r=0.699$, $n=86$, $P<0.001$, where r=correlation coefficient, n=number of observations and P=probability). The reduced major axis (see fig. 1) gives a ventilation equivalent for oxygen of about 2.29 litres of air breathed for each 100 ml. of oxygen consumed (though of course the O_2 consumption of the baby at rest is only about 25 ml./min.). Figure 1 also shows that, as might be expected with the new-born baby, there is considerable scatter between individual observations. It is also noticeable that figures for the premature infants tend to lie above the line, i.e., they need to breathe more for each unit of oxygen they obtain. This is to be expected, as the total volume of ventilation is being measured, and not the alveolar ventilation. The effect noted here is much more pronounced if one examines the data from much smaller creatures, such as the new-born rabbit. Adamsons (1959) found that the ventilation equivalent for 1 ml. of O_2 was as high as 45 ml. at birth; it fell to about 29 ml. in rabbits a few weeks old.

a. Acidosis

It is instructive to examine the data available for the new-born baby to see how far he conforms in detail with the adult. There are satisfactory data for the new-born baby only in respect of respiratory acidosis. Both the premature and the full-term new-born infant show a clear response to an excess of CO_2 in the inspired air (Cross, Hooper & Oppé, 1953). Metabolic acidosis has been studied once in a seven-week-old hydrocephalic infant (Stapleton, Cranston, Cross & Trythall, 1955). I know of no studies of respiration where the infant has been artificially heated.

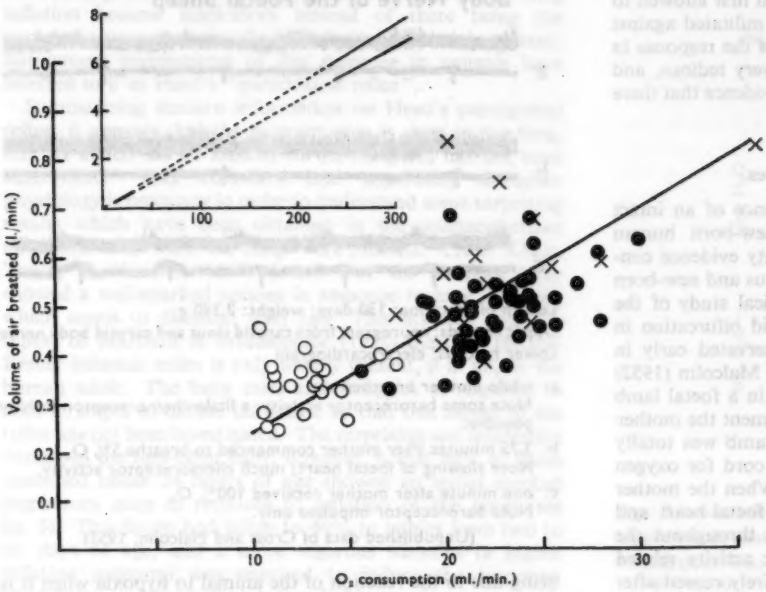
b. Alkalosis

The respiratory behaviour in alkalosis is less clear. High concentrations of oxygen, when given for a period of some minutes, cause hyperventilation both in the adult and in the full-term infant (Cross, 1954). This hyperventilation leads to CO_2 wash-out in the adult, as was deduced by Dautrebande & Haldane (1921). The infant given a high-oxygen mixture also probably induces a state of respiratory alkalosis by over-breathing, since, when the oxygen is withdrawn, there is a very marked hypoventilation for 2–3 minutes. The single infant studied by Stapleton *et al.* (1955) also showed hypo-ventilation when a large dose of sodium bicarbonate was administered, giving rise to metabolic alkalosis.

c. Oxygen Lack

The general picture of respiratory control in the new-born infant with established breathing fits well with that in the adult, as far as CO_2 and pH are concerned. The data for respiratory control of the partial pressure of oxygen in the arterial blood require careful consideration. In the adult, arterial tension of oxygen is controlled reflexly: a lowering of pO_2 stimulates the chemoreceptors of the carotid body and the aortic body. This gives rise to an increase of chemo-receptor impulses passing up the 9th and 10th cranial nerves,

FIG. 1. Relationship between O_2 Consumption and Volume of Air Breathed



○: 21 premature babies
●: 47 full-term babies
(data of Cross, Tizard & Trythall, 1957)
×: 18 babies (both premature and full-term)
(reported by Cook, Cherry, O'Brien, Karlberg & Smith, 1955)

The line through the points is the calculated reduced major axis (Kermack & Haldane, 1950). The slope is 22.89 S.E. ±1.79.

The inset shows the calculated line for the babies and normal limits for the adult, with the limits of the slope set at 21 and 25 (i.e. between 2.1 and 2.5 litres of air breathed for each 100 ml. of O_2 consumed).

causing stimulation of the respiratory centre and increased activity in the muscles of respiration. Here, the ventilation equivalent for O_2 would rise, giving a steeper slope than that shown in fig. 1 (but note that the ordinate changed from air to a mixture poor in O_2).

It is probable that the normal adult (human) has some "tonic" chemoreceptor activity when the subject is breathing air, and the partial pressure of oxygen in the arterial blood is about 100 mm. Hg. Thus, Dripps & Comroe (1947) found some fall of minute volume of ventilation in the human subject when given 100% O_2, in a proportion of adults examined. Loeschcke (1953) found a significant fall in the volume of ventilation when adults were given 32% O_2 in N_2 to breathe after a control period on fresh air. This gas mixture, which would be sufficient to saturate arterial blood fully and raise the arterial pO_2 above 170 mm. Hg, avoided the complication of secondary hyperventilation mentioned under "alkalosis" above.

Dripps & Comroe (1947) have suggested that one can to some extent study the consequences of chemoreceptor denervation in the intact mammal by noting the immediate effect of the administration of a high-oxygen mixture. If there is some chemoreceptor drive while the subject breathes air, then there will be a temporary diminution in the volume of pulmonary ventilation as the high pO_2 in the arterial blood removes this drive. These authors term this effect "physiological denervation" of the chemoreceptors. Curiously, the integrity of the chemoreceptor system may be less well studied if a low-oxygen mixture is administered, for while on the one hand the lowered pO_2 of the arterial blood may stimulate the chemoreceptors, this same lower pO_2 may also depress the medullary respiratory centre. It is thus possible that the algebraic effect of a lowered pO_2 in the inspired air may be to raise or lower the volume of ventilation, or to leave it unchanged. In each case the chemoreceptor reflex could be intact. (Note that in the adult there is an interaction between O_2 lack and CO_2 excess in the arterial blood, so that the total ventilation is not the simple arithmetic sum of each stimulus acting separately (Nielsen & Smith, 1951; Lloyd, Jukes & Cunningham, 1958). That this does not appear to be true in the baby may be owing to a physiological difference, or to the larger effect of environmental temperature on the smaller organism, since this effect has not been adequately estimated in the experiments reported by Cross in previous papers.)

The true extent of the efficacy of the chemoreceptor reflex could only be judged by once again administering a high-O_2 mixture after the low-O_2 mixture, thus revealing temporarily the activity of the medullary centre without chemoreceptor stimulation.

Cross & Warner (1951) and Cross & Oppé (1952) have shown that in full-term and premature infants there is some unsustained increase in the minute volume of ventilation with 15% O_2, and a fall in volume of ventilation when 100% O_2 is breathed after breathing air. This fall is much greater if the 100% O_2 is given after breathing the 15% O_2 mixture. The relevance of these results to the baby at birth has been doubted by Miller & Behrle (1954) and by Miller & Smull (1955), on the grounds that the babies studied by Cross and his colleagues were not truly "new-born", i.e., were not in the first 24 hours of life. However, Miller (1954, Table III) has fortunately performed the experiment of giving 100% O_2 or air to each of ten babies less than 24 hours of age, who had been breathing a mixture of 12% O_2 for five minutes. Both groups show a significant fall in volume of respiration in the first minute of breathing the higher O_2 mixture:

$$t=3.23,\ n=5,\ P<0.05 \text{ for } 100\%\ O_2$$
$$t=3.93,\ n=5,\ P<0.02 \text{ for air,}$$

where $t=t$ test on the differences of the paired observations, and $n=$number of observations, and thus it may be taken that there is an active chemoreceptor reflex in the human infant of less than 24 hours of age. This is of interest, as with

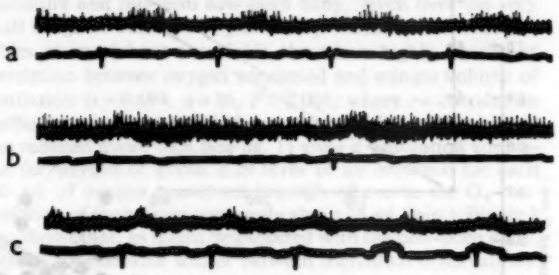
the level of hypoxaemia induced by 12% O_2 the infants have shown a progressive fall in ventilation. They must, therefore, have had some degree of CO_2 retention when first allowed to breathe air or 100% O_2, which would have militated against the hypoventilation. Further examination of the response in the first hour or minutes of life would be very tedious, and there is no reason to suppose from indirect evidence that there would be any other response.

2. Foetal Respiratory Reflexes

While there is thus very suggestive evidence of an intact chemoreceptor reflex in the adult and new-born human being, it is worth examining the rather scanty evidence concerning the chemoreceptor system in the foetus and new-born of other species. Boyd (1937), in his classical study of the development of the structures of the carotid bifurcation in man, finds this area differentiated and innervated early in embryonic life (at about 6 weeks). Cross & Malcolm (1952) recorded carotid sinus nerve impulse traffic in a foetal lamb 20 days premature (see fig. 2). In this experiment the mother was under chloralose anaesthesia, and the lamb was totally dependent on the mother via the umbilical cord for oxygen supply, its own trachea having been tied. When the mother was made hypoxic there was slowing of the foetal heart and continuous firing of the carotid sinus nerve throughout the diastolic period, with no evidence of phasic activity related to the heart beat. This diastolic activity entirely ceased after the mother was given oxygen. While this is not absolute proof of chemoreceptor activity (because one needs for this a single-fibre preparation which responds in no way to blood-pressure changes), it seems highly likely that the cessation of diastolic activity provides strong evidence of chemoreceptor afferent activity in the foetal sheep. This does not tell us that the reflex itself is active, merely that the sensory side of the arc was functional in this foetus at this age. Barcroft & Karvonen (1948) found that cyanide acted as a respiratory stimulant about half-way through gestation in the foetal sheep. They did not show that this stimulant action was abolished by chemoreceptor denervation, but by analogy with known reactions in the adult this seems likely. Dawes & Mott (1959), working on the new-born rabbit, showed conclusively that there was a chemoreceptor reflex which was stimulated by cyanide and abolished by denervation of the carotid bifurcations. They made the further interesting point that, when the new-born rabbit's respiration is increased by breathing a low (11%) oxygen mixture, the stimulation of respiration is maintained if the animal is in a high ambient temperature, but hyperventilation is not maintained if the environmental temperature is only 22° C. These findings, together with those of Hill (1959) in which she showed that moderate hypoxia did not diminish the O_2 consumption of the new-born kitten in a neutral environment, make it clear that the investigations of the effect of 15% O_2 on the respiration of babies (Cross & Warner, 1951; Cross & Oppé, 1952), and the investigation of their metabolism (Cross, Tizard & Trythall, 1955), need repeating with these facts in mind.

In summary, it would appear that the chemical control of respiration in the new-born infant is essentially similar to that of the adult. Although it has been suggested that the respiratory centre of the medulla of the new-born may be more easily depressed by hypoxia, this hypothesis has not yet been fully tested. Many of the results obtained could be interpreted as being due to the reaction of the animal to hypoxia when it is below the critical temperature. The chemoreceptor reflexes, however, are important in the adult for their cardiovascular effects, as well as for responses mediated directly through the respiratory system. There is, as yet, very little evidence on this aspect of chemoreceptor activity in the new-born, but this is a field which might well merit some investigation.

FIG. 2. Neurogram and Electrocardiogram of Mixed Slip from the Carotid Sinus and Carotid Body Nerve of the Foetal Sheep



Length of gestation: 130 days; weight: 2,340 g.
Upper records: neurogram from carotid sinus and carotid body nerve
Lower records: electrocardiogram

a: while mother breathed air
Note some baroreceptor activity; a little chemoreceptor activity possible.

b: 3.75 minutes after mother commenced to breathe 5% O_2
Note slowing of foetal heart; much chemoreceptor activity.

c: one minute after mother received 100% O_2
Note baroreceptor impulses only.

(Unpublished data of Cross and Malcolm, 1952)

3. Lung Inflation in the New-Born

Until recently, little work had been done in the new-born to investigate the reflexes which modify respiration in the adult. Hering and Breuer showed in 1868 that there were sensory areas within the lungs themselves which, when appropriately stimulated, would profoundly modify respiration (Hering, 1868; Breuer, 1868). These responses were destroyed by vagal section. Head (1889) examined the responses of the adult rabbit under chloral anaesthesia to inflation and deflation of the lungs. The rabbit shows slowing of breathing or cessation of inspiratory activity when the intratracheal pressure is increased or the lungs are inflated, and respiration is quickened and more forceful when the intratracheal pressure is reduced. Dawes & Mott (1959) have examined this reflex in the new-born rabbit anaesthetized with pentobarbitone, and find that there are well-marked inflation and deflation reflexes which are destroyed by vagal section.

Head had sought a method of paralysing without irritating the vagus and had cooled it on copper thermodes. This gave a temporary paralysis of vagal sensory function, and he observed that, in the process of recovery, inflation of the lungs gave different responses as the vagi were gradually re-warmed. (Work since that of Head has shown that the temperature at which a particular nerve fibre ceases to conduct is largely a function of the diameter of the fibre concerned.) Head found that with severe cooling the vagi were completely paralysed,

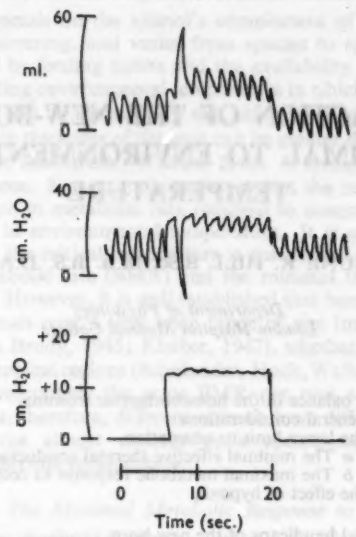
and the animal had slow, deep respiration as though the vagi were cut. At this stage there was no response to lung inflation. As the vagi became a little warmer the response to lung inflation became inspiratory instead of there being the expected apnoea with the inspiratory muscles relaxed. Subsequent investigators of this response in animals have referred to it as Head's "paradoxical reflex".

Summarizing modern information on Head's paradoxical reflex, it appears that the receptor area is deep in the lung, but the actual site or identity of the receptors has not been determined. This excursion into apparently academic physiology is necessary in order to understand some surprising results which have been obtained in the unanaesthetized new-born baby when the lungs were inflated. Cross, Klaus, Tooley & Weisser (1960) found that the sleeping baby showed a well-marked apnoea in response to lung inflation, which seems to diminish with age. This fits well with the report of Marshall & Widdicombe (1958) that the Hering-Breuer inflation reflex is only weakly present, if at all, in the human adult. The baby cries and struggles in response to mild attempts to deflate the lungs, so that this aspect of the reflex has not been investigated. The surprising and interesting finding in the new-born baby, however, is that 80% of those examined under 24 hours of age showed an initial marked inspiratory gasp in response to inflation of the lungs (see fig. 3). This figure had fallen to 40% in babies from two to six days of age, and a more vigorous stimulus (a higher inflation pressure) was required to induce the response. Obviously no rigorous analysis of this gasping response is possible in the new-born baby. From indirect evidence it appears probable that this is an example of the overt appearance of Head's paradoxical reflex in the new-born infant, while in the rabbit or cat it can be elicited only under special circumstances. Dawes, however (in a personal communication, 1960), has found that foetal monkeys, which had been asphyxiated on delivery and were then resuscitated by artificial respiration, came to a point during resuscitation when inflation of the lungs caused an inspiratory effort. This may mean that we have an analogous situation occurring in the monkey, and so this gasping response may now be susceptible to physiological investigation.

The significance of this reflex (if any) can only be a matter for speculation, but certain points may be made. In general, visceral reflexes limit change—thus the normal response to lung inflation is a cessation of inspiratory activity; or the response to a rise of blood pressure is a reflex which limits this rise. In this gasp in response to inflation we have an example of a positive feedback—a change which has occurred is being further promoted. This rather unusual type of response is found in a lung which has just been collapsed (in the foetal condition), and which requires powerful forces for its expansion. While the infant is in a foetal state, it is not only within a fluid-filled system, but is fluid-filled itself. Therefore, pressures applied upon the uterus or by the uterus will be transmitted virtually instantaneously across the infant's body. The stimulus to this gasp reflex appears to be a pressure difference across the lungs. Thus, it appears that this type of stimulus can be applied only after the infant is born.

Bosma, Lind & Gentz (1959) have demonstrated that the new-born infant may make frequent "frog swallowing" movements of air into the fluid-filled trachea at birth. This could conceivably apply the trigger for the large-scale gasp described above.

FIG. 3. Positive-Pressure Inflation of a Male Baby



Age: 2 hours; weight: 4.7 kg.

Upper trace: tidal volume

Middle trace: change in oesophageal pressure (indicating intrapleural pressure)

Lower trace: pressure in the mask

Note that the pressure caused the volume of the baby to increase a little and the oesophageal pressure to become more positive. This was followed by a violent gasp, with a pressure swing of nearly 40 cm. H_2O in the oesophagus. On this occasion the rhythm of breathing was little affected, i.e., there was no Hering-Breuer inflation reflex. (Unpublished data of Cross, Klaus, Tooley and Weisser, 1960)

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REACTION OF THE NEW-BORN ANIMAL TO ENVIRONMENTAL TEMPERATURE

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A Heat balance in the homoeothermic mammal
1 General considerations
2 The lower limit to adaptation
a The minimal effective thermal conductance
b The maximal metabolic response to cold
3 The effect of hypoxia

B Special handicaps of the new-born
4 Effects of immaturity
5 Thermal conductance in the new-born
6 Metabolism in the new-born
7 Conclusion
References

Two recent developments in paediatrics—one, the discovery of the syndrome "neonatal cold injury", the other, the fact that clinicians are revising their views on the treatment of premature infants—have led to a revival of interest in the reaction of the new-born to environmental temperature. An understanding of animal physiology should assist the evolution of the rational approach to these problems. Most adult mammals maintain their deep body-temperature remarkably constant (at between 35° C. and 40° C.—the precise value depends on the species) despite wide variations in environmental temperature. New-born animals seem to be trying to achieve the same end, but, for various reasons that will be discussed later, they encounter special difficulties in doing so. In order to understand these difficulties it is first necessary to consider both the physical and the physiological factors that are involved in the maintenance of the normal homoeothermic state, and to examine their consequences under various circumstances. Then, in the light of these facts, the particular handicaps associated with the new-born state will be discussed.

A. HEAT BALANCE IN THE HOMOEOTHERMIC MAMMAL

1. General Considerations

Over any period during which the body-temperature is constant (regardless of whether or not the body-temperature is normal for the animal in question), the rate at which the animal produces heat is equal to the rate at which it loses it. Heat is produced as a result of metabolic processes; and by far the most important of these is oxidation—non-oxidative processes, for instance glycolysis, yield relatively little heat when an equal amount of food is split. Consequently it is legitimate (and certainly more convenient) to estimate heat production from measurements of oxygen consumption rather than directly by calorimetry. In this paper, heat production has been calculated on the assumption that 1 ml. oxygen yields 5 calories.

Heat is lost as a result of radiation, conduction (usually with mass-flow, i.e., convection) and evaporation; and the rate of heat loss will depend on three main factors: the animal's size, its thermal insulation, and the difference between the temperature of its body and that of the environment. For more detailed information and further discussion see Scholander, Hock, Walters, Johnson & Irving (1950), and Burton & Edholm (1955).

In the homoeothermic animal, body-temperature is maintained constant despite changes in environmental temperature. This state of affairs is illustrated diagrammatically in fig. 1. In the experiment upon which this figure is based, a three-week-old kitten (already a true homoeotherm) showed a variation of body-temperature of less than 0.5° C. over an environmental temperature range of 34–18° C. Though the rate of heat loss is greater when the environment is colder, the homoeothermic animal exactly compensates by an active increase in metabolism. The point at which the active increase first becomes apparent is called the (lower) critical temperature (T_C): above T_C there is a range over which metabolic rate is constant, at the basal (or minimal) level, and this region is called the neutral temperature zone.

The total rate of heat loss, H, is made up of two components: H_{CR}, the heat loss by conduction and radiation; and H_{EV}, the heat loss by evaporation (see fig. 1). H_{CR} is proportional to the difference between the body-temperature (T_B) and the environmental temperature (T_E); and when H_{CR} is given as rate of heat loss per unit surface area (e.g. (cal./m.²)/min.) and T_B and T_E are in °C., then, for environmental temperatures below T_C:

$$H_{CR} = K_{Minimum} (T_B - T_E)$$

where $K_{Minimum}$, the slope ((cal./m.²)/min. per °C.), is the

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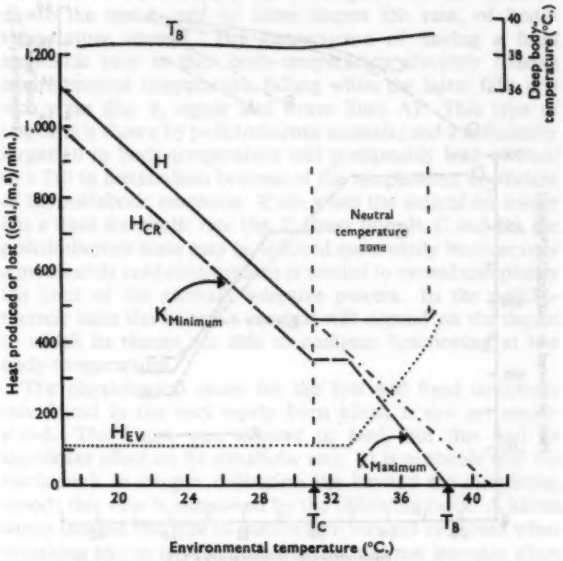
FIG. 1. Diagram Showing Heat Balance Related to Environmental Temperature in a Homoeothermic Animal



H: heat produced (based on measurements of oxygen consumption)
H_{CR}: heat lost by radiation and conduction
H_{EV}: heat lost by evaporation
T_C: critical temperature, i.e., point at which slope of H changes
T_B: animal's body-temperature

The slope of the line H_{CR} gives the effective thermal conductance, K, in (cal./m.²)/min. per °C.: e.g., below T_C this will be $K_{Minimum}$.

effective minimal thermal conductance, i.e., the reciprocal of maximal thermal insulation. The contribution of H_{EV} is clearly indicated by the fact that the line H, when extrapolated, does not intersect the base-line at the animal's body-temperature, but at some point 2.5° C. higher. Below T_C, H_{EV} arises mainly by evaporation from the lungs and from "insensible" perspiration, and in fig. 1, for simplicity, it has been assumed that it is constant in amount. Though this may not be strictly accurate, the error can not be great, and for our practical purposes it can be disregarded. Above T_C the animal first takes steps to increase its thermal conductance (e.g. by an increase in skin blood flow), but when the limit is reached (i.e., K is maximal), H_{EV} must thereafter increase progressively with rise in T_E if the body-temperature is to remain constant. Thus in the upper region of the neutral temperature zone the animal takes active steps to increase H_{EV} by sweating and panting. We shall not be concerned with environmental temperatures above the neutral zone.

2. The Lower Limit to Adaptation

This is entirely determined by two factors, the animal's minimal effective thermal conductance and its maximal metabolic response to cold. In other words, these two factors together determine the lowest environmental temperature at which the homoeothermic animal is yet able to maintain a normal body-temperature; when the limit is exceeded the animal's body-temperature will inexorably fall.

a. The Minimal Effective Thermal Conductance

This depends on the animal's complement of fat and fur or other covering, and varies from species to species. It is influenced by feeding habits and the availability of food; by the prevailing environmental temperature in which the animal lives; and also by the animal's size, since for obvious reasons the absolute thickness of the coat can be greater the larger the animal (see Scholander, Walters, Hock & Irving, 1950). As we have seen, $K_{Minimum}$ alone determines the magnitude of the increase in metabolic rate required to compensate for a given fall in environmental temperature. It is evident from fig. 1 that the critical temperature is determined by both the basal metabolic rate (BMR) and the minimal thermal conductance. However, it is well established that homoeothermic adult animals over a very wide range of size (mouse to elephant, see Brody, 1945; Kleiber, 1947), whether they live in arctic or tropical regions (Scholander, Hock, Walters & Irving, 1950), have roughly the same BMR per unit surface area. In practice, therefore, different animals have different critical temperatures almost entirely as a consequence of their differing thermal conductance.

b. The Maximal Metabolic Response to Cold

It is the maximal heat production per unit surface area that is the other important factor in withstanding cold conditions. Man, and probably most adult animals, can, during maximal exercise, increase their metabolic rate to about fifteen times the basal level. Such a rate of working is very expensive, and in response to a cold environment the maximal metabolic rate that can be sustained is much lower, usually about two to five times the basal level. Generally speaking, the lower figure is found in animals acclimatized to a warm environment, the upper one in cold-acclimatized animals. Just as the BMR per unit surface area is roughly the same for all warm-blooded animals, whatever their size, so it appears that the maximal heat production in the cold per unit surface area is also roughly the same for all homoeotherms, regardless of the animal's size. However, the inevitable consequence of this is that, per kilogram of body-weight, smaller animals have a higher heat production (both basal and maximal) than larger ones.

It is important to understand the reason why large animals are better able to withstand cold environments than small animals. It has been held that this is a consequence of the fact that smaller animals have a greater surface area:weight ratio than larger animals; but this is not a valid argument, because, as has just been pointed out, small and large warm-blooded animals actually have, per unit surface area, a similar maximal metabolic response to cold. This means that the small animals are well able to compensate for their adverse surface area:weight ratio. The true explanation for the increased sensitivity of small animals to cold must lie in the fact that small animals, being small, cannot possibly possess such a thick insulating coat of fat and fur as large animals.

There is still disagreement on the question of how heat is produced in response to cold, that is, whether entirely by muscular activity (shivering and "thermal muscular tone") or partly by other means in addition, e.g. by the uncoupling of biochemical processes either in muscle or in other organs (for further discussion, see Hart, 1958).[1]

[1] See also Hart, *Brit. med. Bull.* 1961, 17, 19.—ED.

3. The Effect of Hypoxia

While oxygen consumption is usually unaffected by increasing the oxygen tension in the inspired gas above that found in the atmosphere (during maximal exercise a small increase can be demonstrated), hypoxia has a pronounced effect. The degree to which an animal's oxygen consumption (and therefore its heat production) is reduced by exposure to hypoxia depends both on the animal's rate of metabolism and on the severity of the hypoxia; the higher the initial metabolic rate, and the lower the oxygen tension, the greater the reduction.

For example, the oxygen consumption of an animal in a neutral temperature environment, metabolizing at the basal rate, is quite unaffected when it is given 10% oxygen to breathe instead of the normal 21%. By contrast, in an environment sufficiently cold to evoke an oxygen consumption greater than the basal rate, the same degree of hypoxia will cause a fall in the animal's oxygen consumption, and consequently also in its body-temperature (Hill, 1958; 1959). Thus a homoeothermic animal, when subjected to hypoxia, will not be able to maintain a normal body-temperature in an environment much below its critical temperature.

B. SPECIAL HANDICAPS OF THE NEW-BORN

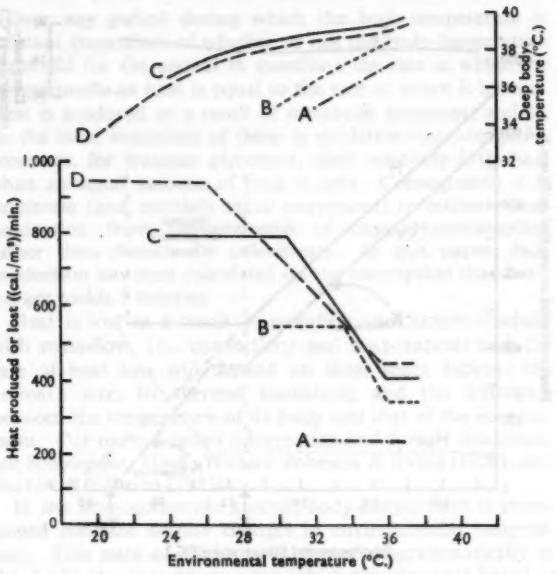
4. Effects of Immaturity

There are striking differences between the "immaturity" at birth of various species of animal. Guinea-pigs immediately after birth are able to fend for themselves; they are homoeothermic and capable of showing a metabolic response to cold similar to that of the three-week-old kitten—demonstrated in fig. 1. New-born kittens on the other hand show a much more limited response—though the degree varies somewhat between different kittens. Figure 2 is a diagram of the heat production and body-temperature at various environmental temperatures of a single originally "immature" kitten on the day of birth and at intervals for the first few days afterwards; compare this figure with fig. 1. New-born rats and mice are apparently even more "immature"—at least to judge by their appearance. It will be a matter of especial importance to discover where, on this scale, human full-term and premature infants fall. The majority of new-born animals have poor thermal control (for further discussion and references, see McCance, 1959) and achieve true homoeothermy at a variable period after birth.

5. Thermal Conductance in the New-Born

Since a new-born animal is so much smaller than its parent, the absolute thickness of its coat is likely to be less than that of the adult. In addition, the complement of fat and fur is greatly influenced by the maturity of the animal at birth. The young of many furred animals are born naked (e.g. the rat), and in the new-born subcutaneous fat is frequently lacking (e.g. pig, McCance & Widdowson, 1959; premature human infant). Consequently, these new-born animals have a relatively high thermal conductance, which decreases rapidly in the first few weeks after birth. Figure 2 (lines B, C, D) illustrates the rapidity of this change in a new-born kitten—note the decrease in steepness of the slope (dH/dT_E) with advancing age. If thermal conductance could have been measured when the kitten was three hours old, it would certainly have been even greater than at two days.

FIG. 2. Heat Production and Body-Temperature Related to Environmental Temperature in a Single New-Born Kitten at Various Ages



A: 3 hours after birth
B: 2 days of age
C: 4 days of age
D: 7 days of age

An additional factor is that all animals are born wet. Until they are dry, their fur not only lacks its proper insulation value, but a substantial loss of heat will occur during the process of evaporation. This point is probably of great importance in connexion with the survival of animals born, not in a nest, but in the open, e.g. lambs.

6. Metabolism in the New-Born

Taking as our example of a newly born immature animal, the kitten whose metabolism in relation to environmental temperature is depicted in fig. 2, we see that its response differs from that of a homoeothermic animal in two important respects.

First, it has, per unit surface area, a lower "basal" metabolic rate; and second, its maximal metabolic response to cold is very limited. The three-hour-old kitten (line A) shows the extreme case—at this age the animal was unable to increase its rate of metabolism in response to a lowered environmental temperature, and its "basal" metabolic rate per unit surface area was about half the adult value. By two days of age its basal metabolic rate had risen somewhat, and it had begun to show a small metabolic response to cold. By the end of the week its BMR had almost reached the adult value; but its maximal heat production in the cold, though very much greater than on the second day, was still low (compare maximal heat production in fig. 2, line D, with maximum attained in fig. 1). However, it is clear that these progressive changes with age will eventually result in the animal's attaining the "adult" type of metabolism.

In the upper part of fig. 2, curves relating deep body-temperature to environmental temperature are shown. These do not necessarily represent the final body-temperature at true thermal equilibrium; but the slope of the line does indicate the trend, and to some degree the rate, of body-temperature change. The consequence of having a fixed metabolic rate is that body-temperature slavishly follows environmental temperature, falling when the latter falls and vice versa (fig. 2, upper and lower lines A). This type of response is shown by poikilothermic animals; and a sufficiently large fall in body-temperature will presumably lead of itself to a fall in metabolism because of the temperature coefficient of the metabolic reactions. Even when the animal no longer has a fixed metabolic rate (fig. 2, lower lines B, C and D), the poikilothermic state may be induced quite easily because only a moderately cold environment is needed to exceed completely the limit of the animal's adaptive powers. In the poikilothermic state the animal's survival will depend on the degree to which its tissues are able to continue functioning at low body-temperatures.

The physiological cause for the low and fixed metabolic rate found in the very newly born kitten is not yet understood. The kitten was allowed to feed, but this had no significant effect on its metabolic rate. It is probable that the bottle-neck in oxygen utilization lies beyond the circulating blood: this view is supported by the following facts. A kitten which showed this type of metabolism was not cyanosed when breathing air; its oxygen consumption did not increase when it was given 40% oxygen to breathe, neither was there any decrease when it was given only 10–15% oxygen. It is tempting to see this low metabolic rate as an intermediate stage in the adjustment from foetal to independent existence. During the later part of gestation a foetus in utero metabolizes at much the same rate, per kg. body-wt., as its mother. (Hasselbalch made a similar statement as early as 1904, and the evidence that has accumulated since then has borne it out; see, for example, Barcroft, 1946; Acheson, Dawes & Mott, 1957; Smith, 1959). The BMR of an adult cat is about 35 (cal./kg.)/min. (Spector, 1956) and that of the three-week-old kitten in fig. 1 is 65 (cal./kg.)/min. (they both have approximately the same basal metabolic rate per m^2.). The metabolic rate per kg. of the three-hour-old kitten, 56 (cal./kg.)/min., is thus intermediate between these two values. If the explanation for this low metabolic rate lies in the persistence of metabolism on a maternal scale, we are in deep but interesting waters; for the physiological mechanism that relates tissue metabolism to body-size is not well understood, despite more than a century of investigation.

7. Conclusion

The available evidence suggests that the metabolism of the new-born of a warm-blooded species often differs from that of the adult animal in two important respects: first, the new-born animal has a lower BMR than is appropriate for its surface area; and second, per unit surface area, the new-born animal has a smaller maximal metabolic response to cold. Thus the heat production of the new-born animal, both minimal and maximal, is lower per unit surface area than that of the adult. The degree of immaturity at birth probably largely determines to what degree the metabolism of the new-born deviates from the adult pattern.

In addition, the new-born animal often has a coat of lower effective insulating power than that of its parent; this is partly because the new-born is smaller than the adult, and is partly a specific attribute of immaturity. This means that, per unit surface area at a given environmental temperature, the new-born animal loses heat much more readily than the adult does.

These facts underlie, and go far to account for, the difficulties which many newly born animals experience in maintaining a stable internal body-temperature.

ACKNOWLEDGEMENT

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PRACTICAL PROBLEMS OF NEONATAL PAEDIATRICS CONSIDERED IN RELATION TO ANIMAL PHYSIOLOGY

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1 Common causes of death amongst the new-born
2 Perinatal asphyxia
3 Experimental anoxia
4 Care of the premature baby
a Oxygen
b Temperature
c Handling
d Humidity, nutrition and medication
References

There is probably no human society so primitive that the care of the new-born infant is determined solely by instinct based on natural selection, but it behoves those who advocate an alteration in traditional practice to justify the preference in terms of physiology and psychology.

Maunsell, in 1838, referring to the custom among Irish midwives of scorching the cord dressing, said:

> It is easy, and, no doubt, appears scientific, to ridicule popular customs and superstitions; but would it not be more discreet and philosophical, to endeavour to discover their origin from some instinct to which they may generally be traced?

The clinician who wishes to base his practice on physiology has two reasons for caution: the first, that the findings in physiology are derived from experiments performed in precisely controlled conditions; the second, that findings in animals do not necessarily apply to man. This is particularly the case with the new-born, because different animals are born at very different stages of development and are adapted to very different environments. However, since one justification of animal physiology is that it should contribute to clinical medicine, whereas the clinician must put his duty to his patient before his wish to contribute to knowledge, it is important that problems in neonatal physiology should at least be formulated in terms of animal experiment before any human infant is subjected to the slightest risk or disturbance for anyone's sake but his own. Were this the rule, not only would ethical considerations be simplified, but it is more than likely that knowledge of the subject would be advanced rather than retarded.

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1. Common Causes of Death Amongst the New-Born

Reliable figures for the total mortality in the new-born period, in some cases going back many years, are known for Great Britain and many other countries.

The position today in Great Britain is that, although only 26 in every 1,000 children born alive die in childhood, more than half of these deaths occur in the first week of life. That this almost certainly does not represent the irreducible minimum, even in the present state of knowledge, is suggested by the lower figures for neonatal mortality in some other countries and the definite differences in Great Britain between different social classes. The precise causes of these deaths are not reliably known; a clearer picture may emerge with the publication of the results of the survey on perinatal mortality conducted by the National Birthday Trust[1] in 1958.

Although the precise proportions are not known, it is clear that most perinatal deaths[2] are attributable to two causes: either environmentally determined asphyxia operating before or after birth, or inability to adapt to extra-uterine life because of premature birth or developmental anomalies. The prevention and treatment of deformity belong to the province of the geneticist and experimental zoologist on the one hand and the surgeon on the other. This paper will, therefore, be concerned with the causes and management of birth asphyxia and the complications of prematurity.

2. Perinatal Asphyxia

Perinatal asphyxia in a potentially viable infant is associated with circumstances in which there is either interference with maternal maintenance of foetal respiration, or damage (asphyxial, iatrogenic or traumatic) to those parts of the organism concerned with independent respiration after birth. This is not to say, however, that perinatal asphyxia can always be predicted or explained.

The obstetrician is warned of the possibility of perinatal asphyxia either by the existence of certain conditions such as toxaemia, prolonged gestation, uterine inertia, obstructed labour, and administration of analgesics or anaesthetics to the mother, or by the detection of certain physical signs in the unborn baby. These signs are (i) changes in foetal heart rate—a sustained rise, or a failure to pick up following a uterine contraction, preceding sustained bradycardia, (ii) the presence of meconium in the liquor, and (iii) violent or absent foetal movements.

A baby should breathe within a few seconds of birth. It is difficult for a clinician to make dispassionate, and therefore accurate, observations when faced with an apnoeic baby at birth, but appearance and behaviour do usually match one of the two patterns traditionally described as blue and white asphyxia. The state of blue asphyxia is characterized by apnoea and cyanosis, but the heart rate is normal or raised, the muscle tone is usually good, and there are often small but reassuring spontaneous movements of the eyelids and lips. In contrast, in white asphyxia there is, in addition to apnoea and pallor, a heart rate below 100/min., invariably poor muscle tone, and no movement either spontaneous or elicitable. These two states could be better defined by further study, in particular of blood pressure (Neligan, 1959),

[1] National Birthday Trust Fund, 57 Lower Belgrave Street, London, S.W.1.—Ed.
[2] Still-births plus deaths of live-born infants in the first week of life.

neurological status (Prechtl, 1960) and biochemical changes (McCance & Widdowson, 1954; James, Weisbrot, Prince, Holaday & Apgar, 1958).

In cases of blue asphyxia the baby can usually be made to gasp by applied stimuli (particularly, perhaps, by pharyngeal aspiration); gasping is usually followed by an increase in pulse rate and forcefulness, and may initiate regular respiration. But if no action is taken, the blue apnoeic baby will usually gasp spontaneously within a few minutes of birth, and this is followed by the same sequence of events. Occasionally the blue apnoeic baby does not respond to stimulation and passes into the state of white asphyxia, from which recovery is extremely unlikely without appropriate treatment. Rarely, asphyxia is not the result of apnoea but of obstruction by a plug of meconium, mucus or blood clot in the upper air passages. The probable sequence of events is hypoxia in utero, inspiratory efforts before delivery, and inhalation of the obstructing material (Emery, 1956; Claireaux, Fraser & Marshall, 1960). In some cases the presence of obstruction may be obvious from the sucking in of the chest wall and failure of the colour to improve with respiratory movements; in others the baby may be apnoeic. If the obstructing material is not rapidly removed it will be sucked down into the lower respiratory tract.

Lastly, it is not uncommon for a baby born by Caesarian section to take a few breaths at once and then lapse into apnoea for several minutes.

Resuscitation

When a baby does not breathe at birth, the attendant is faced with the problem not only of deciding what action to take, but of deciding whether to intervene at all. If effective resuscitative measures were entirely harmless and straightforward there would be no problem.

Most apnoeic new-born babies eventually breathe spontaneously and survive to be apparently normal children, but this fact cannot be taken to justify inaction in cases of neonatal asphyxia.

It is difficult to prove not only association between birth asphyxia and brain damage, but also the causal relationships. Nevertheless, clinical experience, which involves the unconscious assessment of many factors, does strongly suggest that severe birth anoxia sometimes causes severe brain damage (Polani, 1959). The extent to which lesser anoxia causes lesser brain damage is even harder to determine, since neither variable can be accurately measured. Prechtl and Dijkstra (quoted by Prechtl, 1960) have shown that common occurrences which might lead to perinatal hypoxia are significantly associated with minor motor and emotional disturbances in later childhood.

As in other emergency situations in which emotions are liable to vitiate judgement, it is as well to have a set routine for the resuscitation of the apnoeic infant. In blue asphyxia the baby's life is not in immediate danger, and it is usual practice to try to initiate regular respiration by the application of peripheral stimuli or the administration of stimulant drugs. The efficacy of the latter is uncertain in theory and in practice but, when indicated, the value of antagonists to morphine and its analogues is undisputed.

In contrast, in white asphyxia it is generally accepted that stimulation will not initiate respiration until some oxygen has been absorbed, and to ensure this it must be introduced into the lungs. There are, however, considerable practical difficulties in safely producing primary expansion of the lungs by artificial means.

Intragastric oxygen has enjoyed a vogue as a means of artificial respiration in the new-born. Its advocates, however, do not distinguish in their publications between apparently successful applications of the method in which respiratory movements have preceded an improvement in colour, and those in which an improvement in colour precedes the onset of respiration. In the former case, the stimulation caused by passage of the gastric tube or inflation of the stomach has presumably been responsible, rather than the passage of O_2 across the intestinal wall. An improvement in colour preceding respiration is seen occasionally. In our experience this has taken place in cases in which the lungs had already expanded and in which apnoea had supervened, and it is therefore possible that O_2 was being mainly absorbed through the lungs by diffusion.

In addition to measures likely to improve oxygenation there are others calculated to prolong survival, and in certain circumstances this might allow time for resuscitation. Infusions of buffer to raise the pH, though theoretically valid, would hardly be of practical use in the immediate problem of resuscitation, since their administration would occupy the whole attention of a doctor who would be better employed in administration of oxygen. Cooling really might be of value in an emergency, while an untrained assistant waits for help (Westin, Miller, Nyberg & Wedenberg, 1959). This is a subject which deserves further study both in animal and human new-born.

3. Experimental Anoxia

It is of some interest to consider the accepted practice in the care of the asphyxiated new-born human infant, in the light of physiological findings in animals.

There is a vast literature on experimental anoxia and asphyxia, and a study of the relevant contributions (Fazekas, Alexander & Himwich, 1941; Greenfield & Shepherd, 1953; Reynolds & Paul, 1958; Dawes, Jacobson, Mott & Shelley, 1960; Davis, 1961) reveals that there is a certain consistency in the general pattern of the response, despite variations depending on species, age from birth, maturity, and the experimental conditions. At all ages, in most species, and whatever the protocol of the experiment, asphyxia or anoxia is followed in under a minute by a "crisis" characterized by apnoea, bradycardia and loss of muscle tone. Acute anoxia does not appear to cause tachycardia, but this has been described in hypoxia (Born, Dawes & Mott, 1956). Before the crisis there is usually increasing dyspnoea, and after the crisis the apnoeic period is followed by regular and spontaneous gasping. In the foetus the respiratory pattern is necessarily less clear, since it is not certain if the healthy foetus exhibits spontaneous rhythmic respiratory movements in utero; but during asphyxia both apnoea and gasping are seen and it is reasonable to suppose that the order of events is the same as in the new-born animal.

In the rabbit, with the onset of apnoea the pulse slows and the pulse pressure increases; the mean blood pressure, at first well maintained, later falls steadily; the muscles relax; spontaneous movements cease; and the colour changes from livid to pale. During the apnoeic phase, cutaneous stimulation will generally elicit a single gasp, whereas after spontaneous gasping

has ceased, this reaction is absent. Adrenalin injected after the crisis is without effect on pulse or blood pressure. If any oxygen is admitted to the lungs there is usually an increase in blood pressure and heart rate, with re-establishment of rhythmic respiration; and the normal pressor response to adrenalin is restored. It should be mentioned here that the addition of CO_2 does not augment the effect of oxygen administered to the asphyxiated dog (Eastman, Dunn & Kreiselman, 1938) and that, in a number of animals, oxygen given into the stomach has proved ineffective (Cooper, Smith & Pask, 1960; Coxon, 1960). In the absence of resuscitation with oxygen, pulse and respiration fail, but a combination of cardiac massage and artificial ventilation will often restore to life an animal apparently dead. Usually, gasping ceases before the pulse fails but, rarely, the order is reversed (Swann, Christian & Hamilton, 1954; Cassin, Swann & Cassin, 1960). In a young rabbit under light barbiturate anaesthesia and with a body-temperature of 35° C., apnoea may last 10 minutes, gasping may continue for a further 20 minutes, and revival may still be possible after 40 minutes (Davis, unpublished). This prolonged survival is of course characteristic of the new-born animal and it is considered at length elsewhere in this number of the Bulletin (Mott, 1961). In the present context it is necessary to draw attention to the influence on length of survival of temperature, nutrition, and maturity (Britton & Kline, 1945). Miller & Miller (1954) have shown that the survival of the anoxic new-born guinea-pig is considerably prolonged by exposure to low environmental temperatures.

Finally, it is necessary to consider to what extent during anoxia some tissues may have greater resistance than others: tissues like those of the central nervous system are known to be sensitive to lack of oxygen and glucose and may be damaged by anoxia, though the animal survives. For example, Becker & Donnell (1952) and Hurder (1953) compare the learning abilities of animals subjected to prolonged anoxia at birth compared with those of controls not so treated. There is also histological evidence that foetal guinea-pigs (Windle, Becker & Weil, 1944), which are of course relatively mature at birth, and monkeys (Ranck & Windle, 1959), may sustain brain damage in anoxia.

As regards asphyxia in utero, experimental work has so far only underlined accepted practice in obstetrics. It is clear that sustained slowing of the pulse will usually mean anoxia, but it is not certain whether slowing also occurs in hypoxia; and the exact relationship of tachycardia to hypoxia needs to be worked out in greater detail (Hon, 1960). Further information is needed on the response of the foetus to moderate hypoxia, its capacity to tolerate varying degrees of hypoxia, and whether hypoxia before birth leads to difficulties in adaptation to extra-uterine life. Clinical experience suggests that sustained slowing in the human foetus, as in the rabbit, is a feature of anoxia rather than of hypoxia, and its occurrence is a warning that without restoration of effective respiration death will be inevitable within a certain period. In these circumstances restoration of effective respiration is probably best achieved by immediate delivery; the benefit likely to be derived by increasing the concentration of oxygen breathed by the mother is, both in theory and in practice, uncertain (Prystowsky, 1957).

In many respects the rabbit is born at the same stage of maturity as the human full-term infant, so that a comparison between experimental anoxia in the new-born rabbit and naturally occurring birth asphyxia in the human baby may draw attention to real correspondences. Thus there are similarities on the one hand between asphyxia livida in the infant and the apnoea that follows the anoxic crisis in the rabbit, and on the other between asphyxia pallida in the infant and the apnoea that succeeds the phase of gasping in the rabbit; and, if these are real, it might be expected that stimulation would induce gasping in the first instance but not in the second, that spontaneous recovery is likely in the first case but not in the second, and that in asphyxia pallida the need for effective artificial ventilation to prevent circulatory failure is urgent. Thus experimental findings support accepted practice. The fact that anaesthesia tends to prolong the period of pre-terminal apnoea in the rabbit suggests that apnoea in an infant subjected to drugs taken by the mother will probably be prolonged, but that it is likely to be succeeded by spontaneous gasping and to respond to the appropriate antidote. This relation between anoxia and drugs, each augmenting the effect of the other, may explain why in some cases infants will breathe straight away despite heavy maternal sedation, whereas in others there is apnoea that responds to antidotes when only small doses have been given. Weatherall (1960) has supplied evidence that the reaction of new-born animals to various drugs and anaesthetics differs from that in the adult animal.

4. Care of the Premature Baby

It is well known that the baby can initiate respiratory movements from early in foetal life, both in the uterus and after premature birth. The immediate cause of failure of viability in the case of the baby born in the first two trimesters of pregnancy appears to lie in the minute anatomical structure of the lungs. No method currently used will help these infants to survive, and it seems likely that this would only be achieved by the continuance by artificial means of conditions closely resembling the intra-uterine life. The development of extra-corporeal circulations, including devices to function as lungs and kidneys, makes it certain that attempts will and must be made to construct artificial placentas. Experiments have been carried out on the perfusion of animal foetuses (Westin, Nyberg & Enhörning, 1958).

In considering the routine care of the premature infant, two sets of data have to be kept in mind: the first, that which yields indications of the environmental limits inside which such organisms are capable of homoeostasis; the second, that which details the actual causes of morbidity and mortality amongst them. Awareness of the limited powers of the small infant to adapt himself to his environment has led to the custom of nursing the feeble and premature infant in an incubator in which at least the ambient temperature, the oxygen tension, and the humidity can be controlled by the attendant. The ingenuity of the manufacturers of this equipment has for the moment outstripped that of their users; for it is by no means certain what is the ideal environment for the sick or premature baby. To ensure such an environment is vital for the baby in difficulties; for the rest, established practice seems good enough to ensure maximal survival and minimal morbidity—though it is worth pointing out that it may be of great importance for the total development of the human infant that he comes into the right physical relationship with his mother in the first days of extra-uterine life. Such considerations, of course, must always be subordinated to—but need to be reconciled with—the duty to ensure that the physical basis of existence is not endangered.

The major causes of morbidity and mortality among premature infants have been listed by Claireaux (1958). His statistics suggest that most deaths are due to respiratory insufficiency; and in this paper other causes, with their prevention and treatment, will be dealt with cursorily or not at all.

Clinically, respiratory insufficiency usually takes one of two forms: spells of apnoea (Illingworth, 1957) or progressive dyspnoea. Pathologically, the main finding is alveolar atelectasis (Rogers & Gruenwald, 1956), which in progressive dyspnoea is associated with oedema, haemorrhage and the appearance of hyaline membranes in the alveoli and particularly in the alveolar ducts and respiratory bronchioles (Blystad, Landing & Smith, 1951). The findings—clinical, clinico-pathological and morbid-anatomical—in cases of progressive dyspnoea have been the subject of many reviews, of which the most recent are those of James (1959), Cook, Barrie & Avery (1960) and Tizard (1960). In the present context, it is enough to say that, until the cause of the condition is understood, rational treatment must be directed to mitigation of its effects.

In the section that follows, the care of the healthy and that of the distressed new-born premature infant are considered together. In distinction to the situation as regards acute anoxia, the respiratory distress syndrome (progressive dyspnoea) has not yet been reproduced experimentally in animals (except possibly in the thoroughbred race-horse (Mahaffey & Rossdale, 1959), which is not likely to become popular as a laboratory animal) and, in consequence, much of what follows concerns studies carried out on the human infant.

a. Oxygen

In view of the known dangers of exposure to high oxygen concentrations, there is everything to be said for nursing the obviously healthy premature infant in air; indeed, Engelson, Rooth & Sjöstedt (1959) have shown that in the full-term baby the routine use of 15% oxygen is not obviously detrimental. In assessing the comparative advantages of high, normal and low oxygen concentrations, their effect on the incidence and mortality of spells of apnoea needs to be established by a controlled clinical trial. Premature infants often exhibit periodic respiration which disappears when the oxygen content of the inspired air is increased (Wilson, Long & Howard, 1942); but this mode of respiration is perfectly efficient as regards gas exchange and cannot be thought of as pathological unless it leads to spells of apnoea. As an indication for giving oxygen, the skin colour is a poor guide. It is not known whether in the premature infant restlessness is a sign of anoxia, as has been shown to be the case in the older baby with bronchiolitis (Morrison, 1955). Most experienced clinicians would regard retraction as a sign of distress, but the meaning of tachypnoea alone is less clear. In the case of the sick infant in obvious respiratory distress, it is extremely difficult to withhold oxygen, and the practical problem is to determine the ideal concentration to be used for a particular baby at a given moment. Since high oxygen concentrations are known to predispose to retrolental fibroplasia and possibly to cerebral palsy through their effects on the retinal and cerebral vessels (Patz, Hoeck & De La Cruz, 1952), and because there is also evidence that they damage the lungs, decreasing diffusion capacity (Ernsting, 1961), predisposing to collapse and leading to hyaline membrane formation (Bruns & Shields, 1954; Buckingham & Sommers, 1959), this is a question of very great practical importance. That the use of high oxygen concentrations may be beneficial, at least in some circumstances, is suggested by the reported increase in mortality among premature babies following the institution of a rule limiting the maximum permitted concentration to 40% (Avery & Oppenheimer, 1960), but it is of course dangerous to make such comparisons over consecutive periods. Even were there a simple method for bedside estimation of arterial oxygen tension, the estimations would have to be frequently repeated, with much potentially harmful handling and disturbance of the patient. It may be possible to estimate the need for oxygen in another way—by observing the infant's capacity to maintain body-temperature when the environmental temperature is lowered to just below the neutral zone. The findings of Hill (1959) in animals and of Brück, Brück & Lemtis (1958) in babies indicate that even quite immature mammals will increase their oxygen consumption in order to maintain body-temperature, provided that sufficient oxygen is available. Failure to respond in this way might, therefore, be a sign of respiratory insufficiency and suggests the need for increasing the ambient oxygen concentration. Work is needed on the relation between environmental temperature and oxygen consumption in small and distressed infants. The other consequence of asphyxia, hypercapnia, may be as damaging as hypoxia; for instance, an increase in cerebral blood flow caused by CO_2 retention might be responsible for the known complication of intraventricular cerebral haemorrhage. It is not known whether administration of oxygen may aggravate CO_2 retention in the baby. However, for this and other reasons, mechanically assisted respiration might be more logical treatment than a high ambient oxygen concentration (Donald, 1954).

b. Temperature

One aspect of temperature control—the relation of temperature to oxygen consumption—has already been considered. In this connexion it is not yet clear whether, as suggested by Cross, Tizard & Trythall (1958), an infant suffering from hypoxia may respond by lowering his oxygen consumption, with a consequent fall in body-temperature. If this were the case, attempts to raise body-temperature by heating the incubator might interfere with an aspect of homoeostasis. It is clear from the work of Lacomme, Chabrun, Boreau & David (1954), Wagner (1958), and many others, that premature infants can survive and indeed thrive when kept at very low body-temperatures for long periods. In the only controlled study—that reported by Silverman, Fertig & Berger (1958), who compared two groups of babies maintained at temperatures of 28.9° C. and 31.7° C. respectively—mortality was considerably lower in the group at the warmer temperature, partly as a result of an excess of deaths in the other group from haemorrhage. This study cannot be said to close the question, since it relates only to arbitrarily chosen temperature differences applied as a routine measure to unselected premature infants. However, it points the need for caution in future studies, particularly in view of the observations of Haass (1931) on the correlation of blood sugar and body-temperature in the baby, and of McCance & Widdowson (1959) on the metabolism of new-born piglets.

c. Handling

It has been customary to nurse premature babies naked in an incubator so as to facilitate observation and minimize the handling necessary for changing and other nursing procedures.

The young infant, however, is subject to violent reflex actions in response to movement, noise and vibration, and possibly pressure, and if not restrained may as a result move about in the incubator and suffer much disturbance that might be avoided were his movements restricted—one movement leading to another. The naked infant is also exposed to radiant heat and this could interfere with his temperature control (R. H. Mottram, personal communication). Measurements of these effects and controlled trials of swaddling might give interesting results.

d. Humidity, Nutrition and Medication

The minute volume of respiration is increased in respiratory distress (Karlberg, Cook, O'Brien, Cherry & Smith, 1954), and reducing the difference in humidity between inspired and expired air will reduce what might otherwise be excessive water loss (Hooper, Evans & Stapleton, 1954). Water loss has not been estimated in respiratory distress, but as a practical measure Silverman & Blanc (1957) have shown that an increase in humidity promotes the survival of premature babies.

Premature infants are usually starved in the first two days of life, partly because full-term infants are naturally starved until the onset of adequate lactation and partly because of the fear of aspiration. Aspiration does not occur in a well-run nursery for the premature and there are theoretical reasons for thinking that it might be wise to supply distressed premature babies with external sources of carbohydrate. Usher (1959) claims that the administration of glucose and alkali to distressed premature infants reduces hyperkalaemia and mortality. It could be of very great interest to have some information on the glycogen reserves of the human foetus in relation to maturity, birth experience and the health of the mother.

Premature babies are well known to be susceptible to infection, but asepsis rather than antisepsis is desirable, particularly because of the damaging effects of certain drugs, probably resulting from immaturity of mechanisms of detoxication and elimination (Vest, 1958; Odell, 1959). Since the premature infant is often lacking in circulating antibodies, which normally pass from the mother across the placenta towards term, the giving of pooled γ-globulin might be of some value and deserves a clinical trial.

In the treatment of acidosis and hyperkalaemia the administration of (i) glucose and insulin, (ii) digitalis, and (iii) buffer solutions have been advocated. Dawes (1960) has shown that in experimental neonatal anoxia in sheep and monkeys the giving of glucose and sodium lactate in combination, but not singly, will prolong life, but the effect of this treatment in prolonged hypoxia has yet to be explored in animals.

Nothing has been said of many aspects of the care of premature infants, sometimes because too much, sometimes because too little, is known for discussion to be profitable. The above brief review is written in the hope of bridging some of the gaps between practice and theory in care of the newborn infant, and particularly of pointing to situations requiring further study either in physiology or in medicine.

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DR R. A. MCCANCE is Professor of Experimental Medicine in the University of Cambridge. Notes on his earlier work have appeared with papers published in the Bulletin jointly with Dr E. M. Widdowson (*Brit. med. Bull.* 1951, 7, 297; *Brit. med. Bull.* 1957, 13, 3) and with W. L. M. Holman (*Brit. med. Bull.* 1956, 12, 27). In recent years Dr McCance has continued his work on the physiology of infancy and has published papers on this subject and on the effects of prolonged undernutrition in suckling animals. In 1959, he delivered the Leonard Parsons Lectures in Birmingham, on the subject of homoeostasis in the newly born, and in 1960 was awarded the Conway Evans prize by the Royal College of Physicians and the Royal Society. He is a Fellow of the Royal Society, and an honorary member of the Association of American Physicians, the British Paediatric Association and the Schweizerische Gesellschaft für Ernährungsforschung.

MR N. A. MITCHISON is Lecturer in Zoology at the University of Edinburgh. After graduating from Oxford in 1948 he worked for four years under the supervision of Professor P. B. Medawar on tissue transplantation immunity. He then held a Commonwealth Foundation Fellowship at Bloomington, Indiana, and the Jackson Memorial Laboratory, Bar Harbor, Maine, USA, before returning to Edinburgh in 1954. Since then, he has spent periods of research at the Czechoslovak Academy of Sciences in Prague and at Harvard University. He recently held a Visiting Professorship in Genetics at Stanford University, California. Mr Mitchison is interested in the cellular aspects of immunity, and his most recent work has been on the mechanism of tolerance and paralysis. His publications include: "Passive transfer of transplantation immunity" (*Proc. roy. Soc.* B, 1954, 142, 72), and "Studies on immunological response to foreign tumor transplants in mouse; role of lymph node cells in conferring immunity by adoptive transfer" (*J. exp. Med.* 1955, 102, 157).

DR JOAN MOTT has worked on the circulatory system of the eel, and contributed a chapter on the cardiovascular system to *The physiology of fishes* (1957), edited by M. E. Brown. As a Senior Student of the Royal Commission for the Exhibition of 1851, she studied the distribution of blood flow during haemorrhage (*J. Physiol.* 1953, 121, 80). Since 1953 she has been chiefly engaged in investigations of foetal and neonatal physiology and is now a Research Officer in the Nuffield Institute for Medical Research, Oxford. Her particular interests lie in the circulatory and respiratory fields.

DR D. A. NIXON is a graduate of the University of London (University College) and is now a Lecturer in Physiology at St Mary's Hospital Medical School, London. His principal interests are in the physiology of the foetus. His published work, mainly in the *Journal of Physiology*, includes studies on various aspects of inositol and fructose metabolism and on the formation of foetal fluids and urine. Further work is in progress on the development of renal function.

DR DEREK RICHTER is Director of the Medical Research Council's Neuropsychiatric Research Unit at Carshalton, Surrey; he was previously Director of the Neuropsychiatric Research Centre at Whitchurch Hospital, Cardiff, and Lecturer in Neuropsychiatry in the Welsh National School of Medicine. He qualified originally as an organic chemist at Oxford and then worked for some years under Sir Frederick Gowland Hopkins at Cambridge. His research on adrenaline and acetylcholine led him to develop a special interest in the nervous system, which he studied at the Maudsley Hospital, London. Later he completed his medical qualification at St Bartholomew's Hospital, London, and during the Second World War he was attached to the Neurosis Unit at Mill Hill Emergency Hospital. His recent research has included investigations on schizophrenia and on the biochemical changes in the brain caused by stimulation and by drugs. Dr Richter has edited the following publications: *Perspectives in psychiatry* (1950); *Schizophrenia: somatic aspects* (1957); and *Metabolism of the nervous system* (1957). He also contributed previously to the Bulletin (*Brit. med. Bull.* 1949, 6, 44) a paper entitled "Somatic aspects of mental health and disease".

DR HEATHER SHELLEY graduated in Natural Sciences at the University of Cambridge in 1951 and then spent three years in the Department of Pharmacology, University of Oxford, studying problems connected with acetylcholine metabolism, for the D.Phil. degree. Since 1954 she has been a research officer in the Nuffield Institute for Medical Research, University of Oxford, where, in collaboration with Mr H. B. Parry and Dr G. S. Dawes, she is working on some biochemical problems of pregnant and new-born animals. She is particularly interested in ovine pregnancy toxaemia and in the resistance of immature animals to anoxia. Recently she has spent some time in San Juan, Puerto Rico, where, under the auspices of the National Institute of Neurological Diseases and Blindness, Bethesda, she has been working on pregnant monkeys, in collaboration with Dr Dawes. Her published work has appeared in the *Proceedings of the Royal Society*, B, the *British Journal of Pharmacology* and *Journal of Physiology*.

DR J. P. M. TIZARD has been Reader in Child Health at the Institute of Child Health, London University, and Honorary Consultant Children's Physician at the Hammersmith Hospital, London, since 1954. During the Second World War he served with the Royal Army Medical Corps in North Africa and Europe, and on demobilization was House Physician and Resident Medical Registrar and Pathologist at the Hospital for Sick Children, Great Ormond Street, London. In 1949 Dr Tizard was appointed Assistant Director of the Paediatric Unit at St Mary's Hospital Medical School and Physician at the Paddington Green Children's Hospital, London. From 1951 to 1952 he was a Nuffield Foundation Medical Fellow and studied paediatric neurology at the National Hospital, Queen Square, London, and the Children's Medical Center, Boston, Mass. In 1959 he was the Foreign Lecturer of the Journal of Pediatrics Educational Foundation, at Western Reserve University, Cleveland, Ohio. He has published papers in the *Journal of the American Medical Association*, the *Acta paediatrica, Stockholm*, and the *Journal of Pediatrics*, and was joint editor (with Dr A. Holzel) of *Modern trends in paediatrics*, published in 1958. Dr Tizard has recently spent nine months at the Nuffield Institute for Medical Research, Oxford, working on problems of oxygen consumption in young animals. His present work is that of general paediatrician, with research interests in the new-born and in organic nervous disease.

PROFESSOR W. F. WIDDAS, Professor of Physiology at Bedford College, London, was previously Reader at King's College, London, and from 1947 to 1955 was working with Professor A. St G. Huggett, at St Mary's Hospital Medical School, London, on problems in foetal physiology. He qualified in medicine at the University of Durham in 1938 and served seven years in the Royal Army Medical Corps, returning to academic work in 1947. Professor Widdas is mainly interested in the permeability of cells and membranes to hexoses, but he has also made contributions on foetal growth and renal function in the foetus. Papers published include: "Facilitated transfer of hexoses across the human erythrocyte membrane" (*J. Physiol.* 1954, 125, 163); with F. Bowyer, "The action of inhibitors on the facilitated hexose transfer system in erythrocytes" (*J. Physiol.* 1958, 141, 219); and, with J. Stubbs, "The interrelationship of weight change and coronary flow in the isolated perfused rabbit heart" (*J. Physiol.* 1959, 148, 403).

DR E. M. WIDDOWSON is a member of the staff of the Medical Research Council's Department of Experimental Medicine, Cambridge. She has published two previous articles in the *British Medical Bulletin*, both written in collaboration with Dr R. A. McCance, and biographical notes were published on both occasions (*Brit. med. Bull.* 1951, 7, 297, and *Brit. med. Bull.* 1957, 13, 3). She and Dr McCance completed their work on bread by writing a small book with an extensive historical bibliography, entitled *Breads white and brown, their place in thought and social history* (1956). In recent years she has continued to work with Dr McCance on the physiology of the foetus and infant; she has also completed studies on the effects of normal and accelerated growth on the development and chemical composition of animals, and has investigated the calcium and strontium metabolism of new-born infants.

DR I. MAUREEN YOUNG graduated in physiology in 1938 from London University and worked as routine and research assistant at the Medical Research Council's Blood Transfusion Unit in Sutton, Surrey, from 1939 to 1941. Whilst there, she participated in the development of the citric acid-sodium citrate-glucose mixture for blood storage. From 1941 to 1946 she demonstrated and lectured in physiology at Bedford College, London, and is now Senior Lecturer in Experimental Physiology at the Sherrington School of Physiology, London. Working with Sir Joseph Barcroft, Dr Young's interest in foetal and perinatal physiology was stimulated, and she has published papers on the placental exchange of respiratory gases, the placental transfer of various drugs, the sensitivity of the maternal placental vessels to vasoconstrictor substances and the subsequent influence on the foetus. At present, Dr Young's work includes a study of the placental transfer of amino acids and the uptake and elimination of nitrogen by the foetus, and of the cardiovascular reflexes in the new-born infant and the influence of prematurity and perinatal asphyxia on their activity. Among her publications are: "Citric acid-sodium citrate-glucose mixtures for blood storage" (*Quart. J. exp. Physiol.* 1943, 32, 183), written in collaboration with J. F. Loutit & P. L. Mollison; "Some observations on the mechanism of adrenaline hyperpnoea" (*J. Physiol.* 1957, 137, 374); and, with J. D. Martin, "Influence of gestational age and hormones on experimental foetal bradycardia" (*J. Physiol.* 1960, 152, 1).

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Book Reviews

Marshall's Physiology of Reproduction. Volume I, Part 2

Edited by *A. S. Parkes*. 3rd ed. London: Longmans, Green & Co., 1960. xx + 877 pages; figures; illustrations. 26 × 17 cm. £12

Coping with the deluge of treatises, handbooks and colloquia, published today under multiple authorship, is an occupational hazard of the working biologist. Most of these works are highly selective in material and out of date soon after, if not before, publication. The present book, like its equally worthy predecessors [volume I, part 1 and volume II] in the 3rd edition of *Marshall's*, sets out to be comprehensive if not exhaustive. It will serve both the contemporary need and, as important, the need of future generations of biologists who wish to appraise the growth of the scientific study of reproduction in the first half of the 20th century. The standard of scholarship displayed by the eight distinguished authors is awesome. The book is mainly concerned with the role of the male in the reproductive process, and all the animal phyla are given attention; the birds and the bees justly receive as much space as man and farm animals. Nor is there any restriction as to investigational discipline. Gross and microscopic anatomy, physiology and biochemistry, genetics, ecology and behaviour all receive appropriate representation. Some idea of the extent can be got from the fact that some 5,000 names appear in the author index, while the subject index lists about 1,400 main items. A profusion of excellent figures, many of them photographic, adds lustre to the text.

It is sad that the late Arthur Walton did not live to see this volume published. His wide experience and wisdom find expression in four of the eight chapters, on the anatomy and physiology of coitus, fertilization (with C. R. Austin as co-author), and the structure, metabolism and motility of spermatozoa (two chapters with M. W. H. Bishop). F. H. A. Marshall himself prepared the original material for a chapter on external factors in sexual periodicity, which has been revised and extended by E. C. Amoroso. A. J. Marshall joined the editor, A. S. Parkes, to produce a chapter on hormonal control of avian reproduction. Dr Parkes also contributes a chapter on the biology of spermatozoa and artificial insemination. Hormonal control of reproduction in cold-blooded animals is the subject of a chapter by J. M. Dodd.

There is a price to pay for works of such scope and high distinction. So much time is demanded of busy men for its preparation that the original grand design grows dim. Publication is delayed for years and the volumes grow too big and are divided into parts published at widely spaced intervals. The volumes appear out of numerical sequence and with altered contents. Authors die and their drafts are completed by others. In the present instance, a fourth volume, unforeseen at the start, has become necessary and will spread publication into two decades. That the enterprise has not foundered must surely be due to the patience, vigour and pertinacity of the editor. Marshall was fortunate indeed 50 years ago, not only to launch a new branch of biology with his first edition, but also to achieve this with a single slim volume.

B. A. Cross

Oxygen Supply to the Human Foetus

Edited by *James Walker & Alec C. Turnbull*, assisted by *Clement A. Smith & Donald H. Ba[r]ron*. Oxford: Blackwell Scientific Publications, 1959. (Symposium organized jointly by the Council for International Organizations of Medical Sciences ... and the Josiah Macy Jr Foundation.) xii + 313 pages; figures; plates. 23 × 15 cm. £2 7s. 6d.

The nineteen papers in this book were contributed by obstetricians, paediatricians and physiologists to this Symposium held at Princeton, under the chairmanship of Dr Clement Smith, in December 1957. According to the editorial preface, discussions ranged widely, often far from the subject in hand, but the editors have, by rearrangement and provision of cross-references, made it easy to extract information on any particular subject. The book is, however, marred by a large number of faults, far too many to list in detail. Misprints include "gentic" for "genetic" on p. 57, "gestulation" for "gestation" on p. 62 and "Baron" for "Barron" on the title-page. An obscurity occurs at the bottom of p. 79, apparently by the substitution of "base" for "vagus", and anyone relying on sodium chloride instead of sodium fluoride to inhibit glycolysis, as suggested near the bottom of p. 259, will be disappointed. The figures range from adequate to good, but some, e.g., those on pp. 68 and 69, have been spoilt by over-reduction. Many of the figures and tables are badly positioned several pages away from the references to them; Table I is first mentioned on p. 11, but appears on p. 24. The bibliography of about 330 entries is particularly useful as it includes the titles of papers, but there are discrepancies between the dates given in the text and those in the bibliography.

This book must be accepted for what it is, a record of the facts available and hypotheses current over three years ago. The most depressing aspect of the book is the inordinate amount of labour devoted to investigating the quantity of oxygen present in umbilical cord blood at birth, and drawing conclusions about foetal economy from the answers. Discussion failed to resolve several differences of opinion between those who have worked in this field. This is hardly surprising for, as the editorial note on p. 291 explains, and as subsequent evidence indicates, cord blood at birth is unlikely to represent the *milieu intérieur* of the foetus in utero; the great importance of its study lies, as L. S. James has shown, not in the oxygen levels, which are variable, but in the picture it gives of the biochemical condition of the new-born infant, who is usually grossly asphyxiated at birth.

The most refined determinations of the arteriovenous oxygen differences across either the maternal or the foetal side of the placenta reveal nothing about the amount of oxygen reaching the foetus, unless the blood flow through one or other side of the placenta can be determined simultaneously. Recently, human uterine and umbilical blood flow have been determined (at therapeutic abortion), using an electromagnetic flowmeter, and the uterine and foetal O_2 consumption obtained. Many will question the ethics and details of these procedures, as of the earlier attempts described in this book, to measure uterine blood flow by using a modification of the Fick principle. Knowledge of the uterine blood flow is of limited value in arriving at foetal oxygen consumption, as it is impossible to distinguish the placental from the myometrial moiety. The study of the clearance of ^{24}Na from the intervillous space has yielded interesting comparative data, but the figures deduced for the flow through the intervillous space rest on so many assumptions, notably that of its volume, that they are unlikely to be quantitatively exact.

It is against this background that the plea in the epilogue for more animal experiments must be viewed, and heartily endorsed. The obstetrician presumably wants to know two things about the human foetus: (i) what are the mechanisms governing its oxygen supply over a wide range of conditions in both mother and foetus? and (ii) what is the minimum oxygen supply the foetus can tolerate, and for how long? Answers to these questions are emerging from work on other mammals and there is no reason to suppose that they are inapplicable to *Homo sapiens*. Any discrepancies between observations on man and other mammals must of course be scrutinized, and the reasons discovered, but they will be explicable. If this book encourages co-operation in animal work between obstetricians and physiologists, it will have served a valuable purpose. The sponsors of the Symposium deserve thanks for making its proceedings available to a wider public.

J. C. Mott

The Physiology of the Newborn Infant

Clement A. Smith. 3rd ed. Oxford, Blackwell Scientific Publications, 1959. xii + 497 pages; figures; illustrations. 24 × 16 cm. £4 15s.

Readers familiar with the previous editions of Dr Clement Smith's well-known monograph will welcome this new edition. The rapidly increasing number of papers on all aspects of neonatal physiology, which have appeared during the intervening eight years, is reflected in the considerable increase in textual matter and in the enlarged bibliography of the present edition.

The review begins with two chapters on respiration. The first deals with the characteristics of foetal blood in relation to gas transport and foetal respiratory-type movements as observed in various animal species. The second chapter is concerned with the onset of respiration at birth, neonatal tolerance to hypoxia, and various measures of pulmonary function in the neonate. In the first chapter, as in all those dealing with purely foetal function, it is inevitable that emphasis has been placed on observations made in species other than man. However, in a review intended primarily for physicians, as Dr Smith points out, it is desirable to restrict oneself to human data, and this he does wherever such information is available. But a full understanding of the physiological developments occurring at birth requires knowledge of the pattern of function existing in utero, and the closest approach to this at the moment is a very careful interpretation of information available from the study of comparative developmental physiology.

Clinical summaries appear at the end of each chapter. These give information which may be of immediate application to the treatment of infants at this vulnerable period in their life. Dr Smith does not confine himself entirely to the more recent literature but often discusses it in relation to some of the more interesting earlier observations. In a review as comprehensive as this, it is not possible to analyse the finer details of individual experimental procedures, and when seemingly contradictory data are found in the literature both references are included in order that the reader may reach his own conclusion.

Apart from the extensive chapters on respiration and circulation, others which have been largely rewritten are those dealing with icterus neonatorum and liver function, metabolism, nutrition, renal function, endocrinology and immunology. As in the previous editions, there is no chapter on the nervous system. In recent years important advances have been made in the biochemistry and biophysics of the adult nervous system, and the reviewer would welcome a discussion of the application of these advances to some of the problems of neonatal physiology. Furthermore, the importance of knowing the extent to which the stresses of perinatal life may influence the development of the central nervous system leads one to hope that it may be possible to include such a discussion in the next edition of this classic monograph.

D. P. Alexander

Principles of General Physiology. Volume 2: General Physiology

L. E. Bayliss. 5th ed. London: Longmans, Green & Co. Ltd., 1960. xxiii + 848 pages; figures. 24 × 16 cm. £4 5s.

It is now 45 years since the first edition of this book, by W. M. Bayliss, appeared, and it is over 35 years since the last edition, which had been revised by A. V. Hill and others. In this time enormous changes have taken place in physiology, which has shed the independent disciplines of biochemistry and biophysics, in the same way that pharmacology and histology had separated from physiology before. It might be thought that this had reduced general physiology to a vacuum and that every problem in the analysis of function in living organisms might be treated as a problem in one of the daughter sciences. Dr Bayliss rejects this view.

The large amount of physico-chemical background material, which was such a feature of the earlier editions, has been separated from the main body of this work and appeared in 1959 as volume 1.[1] Volume 2 is complete in itself but it follows on naturally from volume 1, and Dr Bayliss has been able to devote all the space in volume 2 to a discussion of general physiology proper.

The first chapter is devoted to a study of the living cell and its environment. Then follows a chapter entitled "Nutrition", which ranges over the whole subject of nutrition as it is now understood, and of general metabolism. It includes sections on the nitrogen cycle, symbiosis, digestion and respiration, among others. This is the part of the book which appears weakest to the present reviewer, who may well be biassed towards biochemistry. Such a wide view is taken of the subject that one can hardly recognize that the wood is made up of trees. It is almost as if physiology, having given birth to biochemistry, is now unwilling or unable to take cognizance of the enormous progress made in the elucidation of the chemical details of the processes of life, contenting itself with outmoded generalizations.

The third chapter deals with excitability and inhibition and contains a good account of the transmission of nerve impulses. Then follow chapters on the effector organs, which include fascinating sections on electric fishes and bioluminescence, and on the receptor organs. It is curious that, in the section on taste, no mention is made of the large amount of work which has been done on the distribution of the taste thresholds for phenylthiourea and related compounds in man.

The last three chapters are about integrating systems: the transport of metabolites and the circulation of the blood, growth and reproduction, and homoeostasis and control systems. Under integrating systems, hormones are dealt with very briefly, the major part being devoted to nervous systems. There is a good account of the nature of the reaction of oxygen with haemoglobin, but the discussion of the transport of carbon dioxide includes some common errors. Thus the acid dissociation constant of carbonic acid is given as $10^{-6.15}$ on p. 586, but this is not the value of the constant K_{CO_2} as defined in equation 7.1 on p. 587. The reviewer is unable to understand the statement, on p. 586, that the carbonic acid–bicarbonate system by itself plays little part in the transport of carbon dioxide.

It is, of course, easy to find small errors in any book covering such a wide field as this one does. For instance, the statement on p. 705 that, among the mammalia at least, it is highly probable that no two individuals have haemoglobins of quite the same chemical nature, is completely contrary to our present ideas on haemoglobin structure. It is also surprising that there is only a very brief mention of immune and other defence mechanisms of the body; and could not the reactions of inflammation be included in general physiology?

Despite these criticisms, this is a fascinating book and it contains a wealth of erudite information. It will be good reading for students and others in many fields of biology and medicine, and it may persuade physiologists to step back from a consideration of their immediate problems and to ponder about their subject in general. Also it should be mentioned that there is an extensive bibliography of the principal books and review articles about the subjects considered, which will be most useful.

S. P. Datta

[1] For review of vol. 1 see *Brit. med. Bull.* 1960, 16, 172.—Ed.

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Human Genetics: Introduction

The reader of this symposium will soon realize how far the subject has been taken since the basic principles were laid down by Mendel and Galton, but he will realize also how firm are the foundations which they laid. In retrospect it seems amazing that Mendel could formulate his principles without having any knowledge of the chromosomes as the conveyors of genetic material, and the realization that their behaviour in meiosis was not only consistent with but actually illustrated Mendel's principles must have been a wonderful revelation to the biologists of the time. Practical methods for the study of human chromosomes are of very recent origin, but it is clear from the papers in this number of the Bulletin that they have already created a new and fertile field of study in human genetics.

I wonder how many of the readers and indeed of the authors will have actually read Mendel's papers. Although interested in genetics since my student days I confess that I never did so until three years ago, and I found it remarkable that Darwin's *Origin of species*, though he wrote it for the layman, is much more difficult to read than the clear scientific brevity with which Mendel describes his beautifully conceived experiments and the preliminary work which led up to them. It is also remarkable that in spite of this, the first edition of the *Origin* was sold out on the day of publication, while Mendel's work had to wait over thirty years before its posthumous recognition. Admittedly it was written in an obscure scientific journal but, where Darwin wrote for his contemporaries, Mendel was a generation or more ahead of his time, perhaps especially in the application of mathematics and experiment to biology. In the meantime, Galton, with his less experimental approach, was elaborating some of the mathematical principles by which human inheritance is still studied.

In this symposium the paper on the quantitative genetics of finger-print patterns is the most purely Galtonian and perhaps the most scientifically beautiful of all; this was a surprise to me for, to a clinician who comes into genetics through the observation of disease, the study of finger-prints has no immediate appeal. Other glimpses of scientific beauty are here in plenty, notably the chromosome studies and, for instance, the application of refined methods to the study of pseudo-cholinesterase and of galactosaemia, where knowing what to measure and how to measure it reveals the discrete genotypes of Mendelian heredity from data which at first sight might appear to show complex or even multifactorial inheritance. In parenthesis, I do not doubt that the same will happen in hypertension, though here, as in other characteristics which depend on measurement for their diagnosis, we may have to distinguish between the inheritance of "blood pressure", which is probably multifactorial, and the inheritance of "hypertension", which is probably not. The inheritance of mental deficiency which is also touched upon in this volume may be a good parallel.

For the rest, this Bulletin makes fascinating reading and will keep physicians and others up to date with the many lines of approach to human genetics which are at present being exploited. There has of course in recent years been an outburst of enthusiasm for the subject. This is partly owing to the fact that its importance has been disclosed by the great advances in the conquest of environmental disease, but also because new methods such as chromatography and new techniques in biochemistry, enzymology, cytology, tissue culture, serology and mathematics have combined in the elucidation of problems previously insoluble, problems which in the first instance have usually stemmed from the observations of the clinician.

I owe to James V. Neel of Michigan the idea that, with all this concentration of specialized talent and technique upon the subject, the need remains or perhaps emerges for a kind of general practitioner of human genetics who, though not able to use all the techniques himself, can nevertheless maintain a general view of the fields in which they can be applied.

Robert Platt

Dr A. C. Stevenson acted as Chairman of the planning committee for this symposium, and also as its Scientific Editor; we wish to thank him for all the help he gave in both capacities.

HUMAN CYTOGENETICS

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1 Types of chromosomal abnormality
a Mongolism
b Other autosomal abnormalities
c Abnormalities of the sex chromosomes
2 Chromosomal mosaicism
3 Neoplasia
4 Conclusion
References

The first task in human cytogenetics was the definition of the normal chromosome set. Von Winiwarter[1] (1912) and Painter (1923) came very close to determining the diploid number correctly. Both asserted that the diploid number of the female was 48, but they differed regarding the male—Painter holding that the number was also 48, and the sex chromosomes XY, and von Winiwarter that the number was 47 with no Y chromosome. Subsequent investigators supported either Painter or von Winiwarter until 1955. In the following year, however, Tjio & Levan (1956) caused great surprise by reporting that they could find only 46 chromosomes in dividing cells of tissue cultures established from aborted embryos. This was immediately confirmed by the finding of 23 bivalent pairs in first spermatocytes (Ford & Hamerton, 1956). There is now universal agreement that the normal diploid number is 46 in both male and female, and that the male is XY, not XO.

Kodani (1957, 1958) claimed to have found an additional small pair of chromosomes in primary spermatocytes of some men, particularly of the Japanese race. In others, he said there was an extra unpaired chromosome, making a total of 47. Nevertheless he regarded 46 as the basic number and suggested that those extra might be similar to the inert supernumerary chromosomes found in some invertebrate and plant species. It is significant that Makino & Sasaki (1960) found a diploid number of 46 chromosomes in 45 aborted foetuses from Japanese mothers, using either tissue cultures or direct squash preparations. Stern (1959), however, had earlier suggested that Kodani's extra chromosomes might be limited to the germ tract in a manner similar to that known to occur in some invertebrates. Accordingly, the issues raised by Kodani's work cannot be finally resolved until there has been an independent reinvestigation of testicular material from Japanese men.

The reason why the number of chromosomes in a normal human somatic cell can now be asserted to be 46 with such confidence, lies in the use of radically different technical methods. Instead of the closely aggregated, long and frequently entangled chromosomes of the natural phases of mitosis and meiosis, devices are used to shorten the chromosomes and disperse them widely within the cell, so permitting unequivocal counting and the ready recognition of morphological distinctions. Colchicine and its less toxic analogue methyldeacetylcolchicine (Colcemid) suppress spindle formation and consequently accumulate mitoses, shorten the chromosomes, and disperse them in the cytoplasm; exposure of the cells to a hypotonic solution swells them by osmotic imbibition and promotes the separation of the chromosomes, presumably by reducing cytoplasmic viscosity; and squashing between slide and cover-slip, or air-drying of cells adhering to a glass surface, spreads out the chromosomes mechanically, at best leaving them lying in a single plane free of overlaps.

At present there are three general methods in use, all of which involve the short-term or long-term culture of biopsy specimens in order to provide dividing cells in vitro: from bone-marrow (Ford, Jacobs & Lajtha, 1958), skin (Tjio & Puck, 1958; Harnden, 1960), or peripheral blood leucocytes (Moorhead, Nowell, Mellman, Batipps & Hungerford, 1960). Tissue cultures which give satisfactory final preparations can also be established from operation specimens or even from material taken at autopsy. The three methods should be regarded as complementary rather than alternative.

The absolute length and general appearance of the chromosomes vary to some extent according to the source of dividing cells, the length of exposure to colchicine or Colcemid and the fixative and stain used. Nevertheless they retain their individuality and some can be recognized at a glance by the two primary distinguishing features of relative length and centromere position. The longest measures about 7 μ and is about five times the length of the shortest. The centromere may vary in position from median or submedian (metacentric chromosomes) to nearly terminal (acrocentric chromosomes).

Figure 1 shows the karyotype of a normal male, that is, a paired array of the chromosomes from a single diploid cell. The numbering is that adopted by an international study group and referred to as the Denver system (*Lancet*, 1960). The differences between groups are clear, but within some groups the distinctions between individual chromosomes may be insufficient for a valid separation by relative length and centromere position alone.

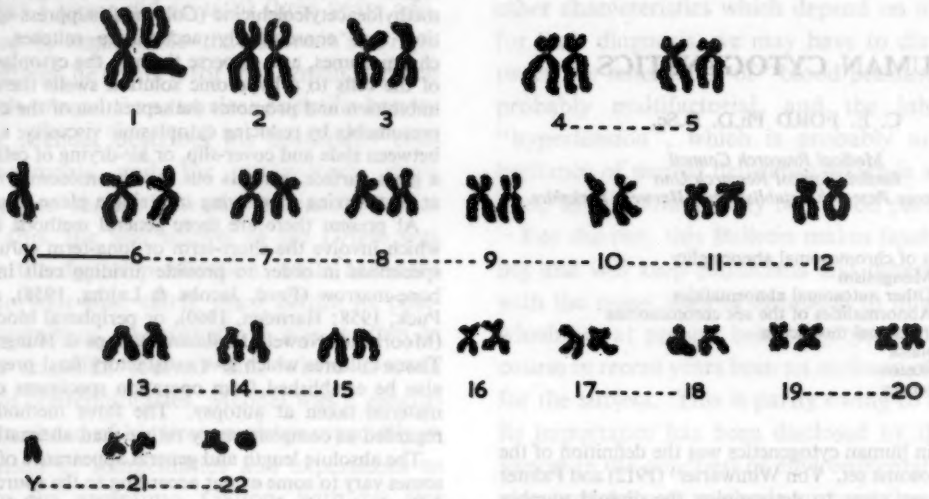
The meiotic chromosomes as seen in primary spermatocytes present a very different appearance. At pachytene the members of homologous pairs lie closely side by side, their longitudinal specificity and exactness of association being revealed by the pairs of darkly staining chromomeres that occur at irregular intervals. Preliminary maps of the chromomere patterns of some of these pachytene bivalents have been made (Schultz & St Lawrence, 1949; Yerganian, 1957). By diplotene and early diakinesis the 23 bivalents have become four-stranded. They are shorter and thrown into loops, successive loops tending to lie in planes at right angles to each other. The nodes, or chiasmata, can be counted, and average about 55 per cell (Ford & Hamerton, 1956). If the chiasmata are interpreted according to the generally accepted view and taken to represent genetic cross-overs, the average genetic length of a human autosome in the male would be about 120 cross-over units.

1. Types of Chromosomal Abnormality

The cytogenetic studies of animal and plant species carried out in the 1920's and 1930's established the types of chromosomal abnormality that arise naturally or can be induced experimentally (Darlington, 1937; White, 1954; Swanson, 1957).

[1] Later known as de Winiwarter.—Ed.

FIG. 1. The Chromosomes of a Normal Human Male



(Feulgen squash preparation from bone-marrow; magnification × 2,500)

The dotted and solid lines indicate the groups in which the individual chromosomes are difficult to distinguish. This difficulty is most pronounced as between the X chromosome and autosome pair no. 6.

Numerical abnormalities or heteroploids are of two types: those in which there is a change in the number of whole chromosome sets (polyploids or euploids, including haploids, triploids, and tetraploids), and those in which the chromosome number is not an exact multiple of the haploid number (aneuploids). Important among the aneuploids are trisomics, in which a single chromosome type is represented three times instead of twice, and monosomics, in which there is only one representative of a given chromosome. Numerical abnormalities arise through mitotic or meiotic errors: (i) lagging of a chromosome on the spindle and subsequent elimination from the daughter nuclei; (ii) non-disjunction, in which both daughter chromosomes (or both members of a bivalent pair) are incorporated in one daughter nucleus; (iii) multipolar spindles resulting in the distribution of the daughter chromosomes between three, or sometimes four, daughter nuclei instead of the normal two; (iv) failure of the spindle to appear, followed by the formation of a single "restitution nucleus" incorporating all the daughter chromosomes; and (v) endomitosis, the replication of the chromosomes without breakdown of the nuclear membrane.

Structural changes are the result of breakage of the chromosomes and rejoining of the broken ends in new ways. The terms used to designate the principal types are self-explanatory. Balanced changes, i.e., reciprocal translocation and inversion, usually have no detectable phenotypic effect. On the other hand the unbalanced changes, duplication and deficiency (or deletion), usually have a recognizable, sometimes severe, phenotypic effect, long ones often being lethal, i.e., leading to death during embryonic development. A rearranged chromosome may be morphologically distinguishable from chromosomes of the normal set but, whether distinguishable or not, it is likely to have the same stability at mitosis as a normal chromosome, provided that it carries a single centromere, and perhaps also two of Muller's hypothetical telomeres (Muller, 1932).

The meiotic divisions of gametogenesis present a different test to rearranged chromosomes. The irregular pairing which then occurs frequently leads to the formation of gametes with the normal haploid number of chromosomes but lacking a particular chromosome segment, or bearing an extra segment, or both. These unbalanced gametes are viable as such, though the almost invariable consequence of their union with a normal gamete at fertilization is the production of an inviable zygote. The frequency of zygotic lethality is particularly high when individuals bearing certain reciprocal translocations breed, their fertility often being reduced to about one half.

Isochromosomes are a special class of structurally abnormal chromosomes, the two arms being not merely morphologically identical, but genetically homologous. They arise by misdivision of the centromere, that is by transverse division at anaphase instead of the normal longitudinal division.

In a further class of abnormal individuals, mosaics, the cells of the body are compounded of cells of two or more distinct chromosomal types. If the gonads are involved the individual is a gonosomal mosaic.

The types of chromosomal abnormality just described are probably of universal occurrence, and it was to be expected that they would arise from time to time in our own species. Before the introduction of the new technical methods and the consequent spate of discoveries it would have been reasonable to hold that most, if not all, numerical abnormalities would be lethal, that reciprocal translocations and inversions would probably be associated with an erratic succession of miscarriages and normal births, and that duplications and deficiencies, if detectable, would mimic dominant Mendelian genes in their behaviour. Today, the position is changed only in that we know that some numerical abnormalities are

compatible with live birth, if not with freedom from congenital defect.

a. Mongolism

The presence of an additional small acrocentric chromosome (probably an extra no. 21 of the Denver system) in typical cases of mongolism was first reported by Lejeune, Gautier & Turpin (1959a, 1959b) and quickly confirmed (Böök, Fraccaro & Lindsten, 1959; Ford, Jones, Miller, Mittwoch, Penrose, Ridler & Shapiro, 1959; Jacobs, Baikie, Court Brown & Strong, 1959). If mongols are indeed primary autosomal trisomics, several of the previously puzzling features of this condition are immediately accounted for. These include its erratic occurrence, the extensive array of minor anatomical stigmata, the invariable concordance between monozygotic twins, and the fact that the few children born to mongol mothers are about equally mongols and normal children (see Warkany, 1960). The striking increase in the frequency of mongol births as the age of the mother rises, reaching 1 in 40 at the age of 45 and over (Penrose, 1954), is not explained. It is reasonable to look for a relationship between this feature and some factor promoting non-disjunction of autosome pair 21 that is dependent on the age of the maturing oocyte.

Familial mongolism occurs more frequently than would be expected from the chance coincidence of independent events. Moreover, in affected families the mothers of mongols tend to be younger than the mothers of unselected mongols (Penrose, 1951), a feature which may account for the relative flatness of the over-all incidence curve before the maternal age of 30. These considerations prompted the investigation of mongols born to young mothers, and led to the discovery of a mongol child with the normal number of 46 chromosomes (Polani, Briggs, Ford, Clarke & Berg, 1960). Though the number was normal the karyotype was not, a member of the 13/15 group having been replaced by one that resembled one of the shorter members of the 6/12/X group. The favoured explanation was that this abnormal chromosome was derived by a markedly unequal reciprocal translocation between a normal member of the 13/15 group and a no. 21, effectively being a compound of the two long arms. By hypothesis, the child would be virtually trisomic for autosome no. 21, like most mongols.

The complementary product of reciprocal translocation would have been composed of the two short arms. Arbitrarily choosing no. 13 as the longer of the two chromosomes concerned, the two products could be designated $13^1/21^1$ and $13^s/21^s$. An individual bearing these two chromosomes, plus a normal no. 13 and a normal no. 21, would have a balanced segmental constitution and therefore would be expected to have a normal phenotype. Were the small $13^s/21^s$ to be lost, leaving only 45 chromosomes, the phenotype might still be normal, since the regions immediately adjacent to the centromeres probably have little genetic activity, if any at all.

Individuals with 46 or 45 chromosomes bearing $13^1/21^1$ would be expected to have entirely normal offspring as well as carriers like themselves. Also, in view of the meiotic irregularities that occur in individuals heterozygous for chromosome rearrangements, there should be a certain output of unbalanced gametes. Union of an abnormal gamete carrying $13^1/21^1$ and a normal 21 would yield a zygote with the greater part of autosome 21 represented in triplicate, and presumptively destined to develop into a mongol child. Other unbalanced zygotic types are likely to lead to miscarriage and possibly even to the failure of the blastocyst to implant. A chromosome like $13^1/21^1$ could therefore be transmitted through several generations without outward sign of abnormality. It would also provide a simple explanation for the familial transmission of mongolism and for the age of the mothers in these families being lower than average at the birth of their mongol children.

These suppositions have been supported by the discovery of a family in which the propositus was a 46-chromosome mongol like the one just discussed. Two sibs, one normal, one mongol, were not examined. The mother had 45 chromosomes, including one like $13^1/21^1$. The maternal grandmother had 45 chromosomes like the mother, and so did a maternal aunt whose only child was a mongol (Carter, Hamerton, Polani, Gunalp & Weller, 1960). In a very similar pedigree, grandmother, mother and daughter each had 45 chromosomes, again including one like $13^1/21^1$, and two mongol sibs of the normal daughter had 46 chromosomes like the other presumptive translocation mongols discussed. The two other pregnancies of the 45-chromosome mother terminated in miscarriage (Penrose, Ellis & Delhanty, 1960).

Other mongol children with 46 chromosomes have also been described (Fraccaro, Kaijser & Lindsten, 1960; Makino, Tonomura & Matsunaga, 1960; Penrose *et al.* 1960). All nevertheless have an abnormal chromosome constitution including, in every case, one chromosome that could be interpreted as a product of reciprocal translocation or as an isochromosome for the long arm of no. 21. The case of Fraccaro, Kaijser and Lindsten, however, has certain puzzling features, and the interpretation of the abnormal chromosome is uncertain.

Clarke, Edwards & Smallpeice (1961) have recently examined the chromosomes of a child in whom the diagnosis of mongolism was equivocal. The child is now three years old and has certain mongoloid features but is said to have normal intelligence for her age. Examination of the blood revealed only cells with an apparently normal set of chromosomes. However, two separate skin cultures proved to be mixtures of apparently normal cells with 46 chromosomes and cells with 47 chromosomes containing the extra small acrocentric autosome typical of mongols, 47% with 47 chromosomes in the one culture, and 62% in the other. The child is therefore inferred to have a mosaic constitution. These observations immediately account for the uncertainty of diagnosis. They also suggest an alternative explanation for multiple mongol births in a sibship, namely that a normal, or at least not obviously mongol, parent might yet be a gonosomal mosaic with a greatly increased risk of having mongol children.

b. Other Autosomal Abnormalities

Two further types of autosomal trisomy syndrome are now recognized. Both involve multiple congenital abnormalities though neither conforms to any previously recognized condition. The phenotypic similarity of seven infants, all of whom had a single additional chromosome (Edwards, Harnden, Cameron, Crosse & Wolff, 1960; Smith, Patau, Therman & Inhorn, 1960), strongly suggests that the same extra chromosome is present in all of them, although the one group identified it as a no. 17 and the other as a no. 18. However, these two chromosomes are very similar and one of the identifications

may have been in error. Low-set, malformed ears and micrognathia are striking features of these cases.

Two trisomic individuals reported by Patau, Smith, Therman, Inhorn & Wagner (1960) and one reported by Ellis and Marwood (personal communication, 1960) also show clear points of resemblance. Here the extra chromosome is a member of the 13/15 group. A dominant feature is serious eye defect, amounting to anophthalmia in one case.

Single examples of four other autosomal trisomic conditions have been claimed, and also two cases in which there is trisomy of one autosome and monosomy of another so that the normal total of 46 chromosomes is retained. If the interpretations are correct, examples should eventually be found by other groups, and it would therefore be well to suspend judgement on these cases for the present.

The most remarkable autosomal abnormality yet discovered was reported by Böök & Santesson (1960a), who found triploid cells with 69 chromosomes in tissue culture from a boy with multiple congenital abnormalities who was a year old at the time of examination. The individual chromosomes matched well in sets of three, except the sex chromosomes, which were consistent with an XXY interpretation. In additional cultures established from skin and fascia lata, a few cells with the diploid number of chromosomes were found as well as a majority of triploid cells, whereas in bone-marrow culture all the cells examined were diploid (Böök & Santesson, 1960b). This case presents several intriguing problems and further reports will be awaited with great interest.

The first case of presumptive reciprocal translocation in man was described by Turpin, Lejeune, Lafourcade & Gautier (1959) in a boy with multiple skeletal defects. Cells in culture contained only 45 chromosomes. Two chromosomes were lacking as compared with the normal male set, a member of group 13/15 and a member of group 21/22. This deficiency was almost restored in a quantitative sense by the presence of an extra medium-sized chromosome whose two arms corresponded closely in length to the long arms of the two missing chromosomes. The expected complementary product of the rearrangement was not detected. The case therefore has strong formal similarities to the individuals with 45 chromosomes in the pedigrees of familial mongolism already discussed.

Two other examples of presumptive reciprocal translocation have been reported. It will be valuable to have information regarding the families of these and any other translocation cases which may be found, particularly the pregnancy histories where one spouse is a carrier.

c. Abnormalities of the Sex Chromosomes

Investigation of cases of disturbed sex development has revealed many types of abnormality of the sex chromosomes. Since they are reviewed elsewhere in this issue,[1] it will suffice to say here that XO, XXX and XXXX types (with 45, 47 and 48 chromosomes respectively) are females, and that XXY, XXXY, XXXXY, and XXYY types (with 47, 48, 49, and 48 chromosomes respectively) are males. Chromosomal abnormality was originally suspected in many of these instances from examination of buccal mucosa smears for the presence of chromatin bodies (Barr, 1959). These, as is well known, are normally confined to females. They are now considered likely to be derived from the whole or part of a single X chromosome that condenses and stains differentially in mitotic prophase (Ohno & Makino, 1961). In the multiple X chromosome abnormalities a substantial proportion of the cells contain more than one sex chromatin body, the maximum being one less than the number of X chromosomes.

Triplo-X women are of special interest in that several are of proved fertility. Assuming a regular two-to-one disjunction of the expected X chromosome trivalent at meiosis, they should produce XX ova and X ova in equal numbers and so give birth to four classes of offspring in equal numbers—normal XY sons and XX daughters, XXY sons with the Klinefelter syndrome and triplo-X daughters like themselves. Yet on the evidence of buccal smears the ten children recorded are all normal (Fraser, Campbell, MacGillivray, Boyd & Lennox, 1960; Stewart & Sanderson, 1960; Barr, personal communication, 1961). The several possible explanations of this departure from the simple explanation include XX/XXX mosaicism involving the ovaries, loss of one X chromosome by lagging in meiotic anaphase, and perhaps lowered viability of XX ova.

Although the occurrence of XY individuals with an essentially female phenotype (Harnden & Stewart, 1959; Jacobs, Baikie, Court Brown, Forrest, Roy, Stewart & Lennox, 1959) shows that possession of a Y chromosome is no guarantee of masculinity, the invariable presence of a Y in normal males and in anomalous individuals with a male phenotype suggests that this chromosome is nevertheless necessary for testicular development. The occurrence of testicular tissue in apparently XX true hermaphrodites can be reconciled with this simple hypothesis only on the basis of undetected mosaicism, such as the XX/XXY type mentioned below (Ford, 1961). It is most desirable that cultures for cytological examination should be set up directly from gonadal tissue of true hermaphrodites when the opportunity arises.

A presumptive deficiency for part of an X chromosome (Jacobs, Harnden, Court Brown, Goldstein, Close, MacGregor, Maclean & Strong, 1960) and three examples of a presumptive isochromosome derived from the long arm of the X (Fraccaro, Ikkos, Lindsten, Luft & Kaijser, 1960) have been recorded. Comparison of the phenotypes of individuals carrying chromosomes like these should eventually lead to an understanding of the action of different regions of the X chromosome.

2. Chromosomal Mosaicism

Chromosomal mosaicism has already been mentioned several times. The phenomenon was first suspected in a chromatin-positive subject with Klinefelter's syndrome whose bone-marrow contained many cells with 46 chromosomes as well as a majority with 47, the two cell types being interpreted as XX and XXY respectively (Ford, Polani, Briggs & Bishop, 1959). Instances of mosaicism are now well established and include three additional types, XO/XX (Ford, 1960a), XO/XY (Hirschhorn, Decker & Cooper, 1960), and the particularly noteworthy XO/XXX case (Jacobs *et al.* 1960). The last example implies origin by non-disjunction at an early mitosis (not necessarily the first, since the embryo proper arises from a portion only of the inner cell mass of the blastocyst). It also suggests that three-component mosaics will eventually be found.

In the XO/XXX case mentioned, mosaicism was demonstrated in bone-marrow and two separate skin cultures, but

[1] See Harnden & Jacobs, p. 206, and Polani, p. 200 of this number of the Bulletin. —Ed.

not in blood. It follows that a mosaic may not be detected by examination of a single tissue or single biopsy specimen. The likelihood of detection will presumably depend upon the time of origin in relation to development, on subsequent migratory movements and on the rates of proliferation of the different cell types, which may not be equal. It is not inconceivable that a cell with a normal chromosome complement might arise in an aneuploid individual after embryogenesis is complete and yet, through differential proliferation, become an important or even dominant component of the tissue concerned.

3. Neoplasia

The study of the chromosomes in neoplastic conditions is a legitimate part of cytogenetics. Most progress has been made with the analysis of the leukaemias (Baikie, Court Brown, Jacobs & Milne, 1959; Ford, 1960b; Sandberg, Koepf, Crosswhite & Hauschka, 1960). In many acute cases cells are present in bone-marrow or blood containing abnormal chromosome sets, sometimes of such individuality and constancy as to indicate that all cells of the abnormal type are descended from a single progenitor. The most remarkable discovery in this field is that cases of chronic myeloid leukaemia very commonly exhibit a characteristic defect (presumptive deficiency) in one of the small acrocentric chromosomes (Nowell & Hungerford, 1960; Tough, Court Brown, Baikie, Buckton, Harnden, Jacobs, King & MacBride, 1961). This is the first instance to be reported in which a specific chromosomal change is characteristic of a specific type of neoplastic disease.

4. Conclusion

The most serious limitation of human cytogenetics is that interpretation cannot be put to direct test, as is possible with experimental material. It is therefore necessary to proceed by the comparison and integration of many individual observations.

Nevertheless, from a clinical point of view it is now clear that gross chromosomal abnormality is a major factor in the causation of errors of sex development. Its contribution to other types of congenital abnormality and to mental defect is proportionately less, though still important. On the basis of present evidence a reasonable estimate for a white population is that about one in 300 live-born children is likely to be affected.

At the same time information of more general zoological interest is being gained. We now have a much better understanding of the chromosomal basis of sex determination in our own species and a likely model for other mammals; knowledge of the effects of autosomal unbalance on development and of the frequency of origin of new chromosomes by structural change is growing; and we may perhaps hope that detailed study of mosaic individuals will prove of value to embryology.

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MONGOLISM*

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1 Clinical observations
2 Incidence
3 Relation to maternal age
4 Familial concentration
5 Cytology
6 Genetics
7 Conclusion
References

For nearly a century, since its first description by Langdon-Down (1866), mongolism has presented some of the most fascinating yet baffling problems in the whole of medical science. In recent years many facts have been discovered and some questions have been very clearly answered; at the same time plenty of problems remain. There is no indication yet as to how the cytology is related to the pathology and no specific therapy has yet been devised. It is evident that the full understanding of the processes involved in this strange condition holds an unexpectedly important place in the biology of the human race.

1. Clinical Observations

In mongolism, physical peculiarities ranging from slight anomalies to severe malformations have been found in almost every tissue and, as time goes on, more of these are described. The general clinical survey of Øster (1953) usefully supplemented that of Carter & MacCarthy (1951) concerning the appearances at birth, and that of Lowe (1949) on the ophthalmology; the presence of a third fontanelle or of small white spots on the iris has been shown to be an important diagnostic criterion. Especially intriguing are the unusual dermatoglyphic patterns on the palms and soles, which have been intensively studied by Cummins (1936) and by Walker (1957), and which are more characteristic than the flexion creases (see fig. 1). Failure of polymorphonuclear neutrophil leucocytes in the blood to mature fully, observed by Turpin, Bernyer & Teissier (1947), has been studied by Mittwoch (1959) in relation to sex. The effect seems to be independent of any reaction to infectious disease. Indeed, the incidence of severe infections in mongolism has been well controlled since the advent of antibiotics. Life tables made for mongols in 1932 indicated an expectation of life at birth of only nine years, whereas, some 15 years later, the expectation had risen to 12 years (Penrose, 1949). Inspection of more comprehensive statistics (Brothers & Jago, 1954; Carter, 1958) suggests that the health of these patients continues to improve. Their liability to infection is likely to be a consequence of abnormal structure in cells, mucous membranes and other tissues rather than any defect of antibody formation, for this has been shown to be satisfactory (Donner, 1954). The prevalence of leukaemia in childhood, which seems to be twenty times greater than in the general population, may be of significance in this connexion (A. Stewart, personal communication, 1960).

Innumerable biochemical investigations have been carried out, and many deviations from the normal have been observed which, however, are not very constant from one case to another. Among the peculiarities found recently are decreased blood-calcium levels (Stern & Lewis, 1958) and diminished excretion of tryptophan metabolites (Jérôme, Lejeune & Turpin, 1960).

In the earliest descriptions, the peculiar mentality of mongols was given prominence, but surprisingly few exact psychological studies have been attempted. Those well acquainted with these patients (Hilliard & Kirman, 1957; König, 1959) have summarized general impressions. Results of exact testing (O'Connor, personal communication, 1960) lead to the view that visual recognition is unexpectedly efficient. Examination of relatively intelligent patients (Stickland, 1954) shows that abstract reasoning is exceptionally retarded.

2. Incidence

In communities of European origin the incidence of mongolism at birth has been frequently measured in maternity hospitals. Thus the figure of 1:776 was found by Malpas (1937) in Liverpool, that of 1:636 by Jenkins (1933) in Chicago, and that of 1:666 by Carter & MacCarthy (1951) in London. The Danish survey covering the whole population (Øster, 1953) gave a value of 1:765 and a comparable, but more extensive, Australian survey (Collman & Stoller, 1961) gave 1:688. There is no definite proof that mongolism is either increasing or decreasing, but there is some evidence that it existed in early historic times in England (Brothwell, 1960). Epidemiological

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HUMAN CYTOGENETICS

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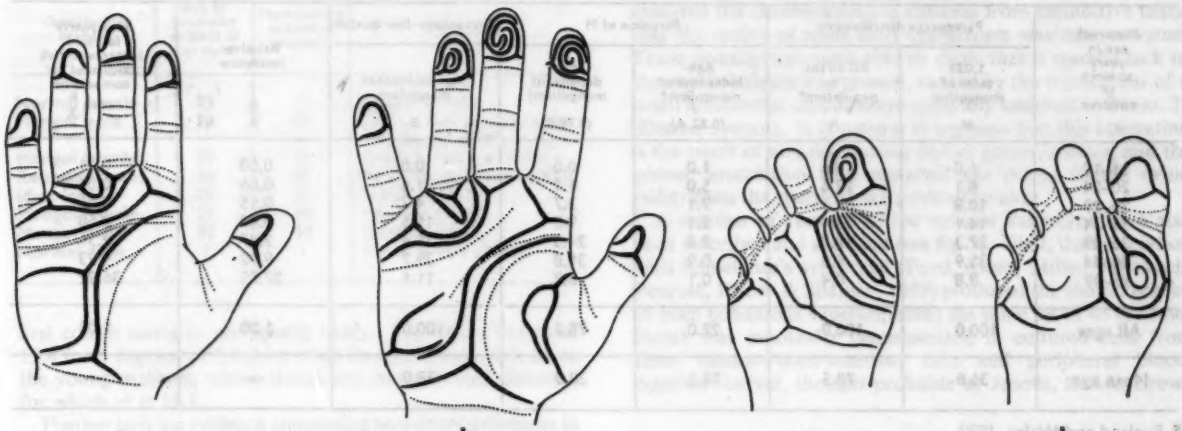
FIG. 1. Diagrams of Main Lines of Palmar and Plantar Dermal Ridges



a

a: palm of mongol patient showing characteristic features:
- i. triradius near centre;
- ii. absence of pattern in thenar area;
- iii. digital loop between digits III and IV;
- iv. radial loop on tip of digit IV;
- v. ulnar loops on all other digits.

b

b: palm of normal subject showing features rare in mongolism:
- i. triradius at ulnar edge of hypothenar region;
- ii. pattern in thenar area;
- iii. numerous whorls on tips of digits.

c

c: sole of mongol patient, emphasizing the hallucal area; a narrow digital loop or absence of pattern is typical.

d

d: sole of normal subject, emphasizing the hallucal area; such a well-developed pattern or a whorl is rare in mongolism.

investigations indicating the possible significance of season and locality (Pleydell, 1957; König, 1959; Collman & Stoller, 1961) have sometimes been interpreted as evidence for the influence of infectious disease.

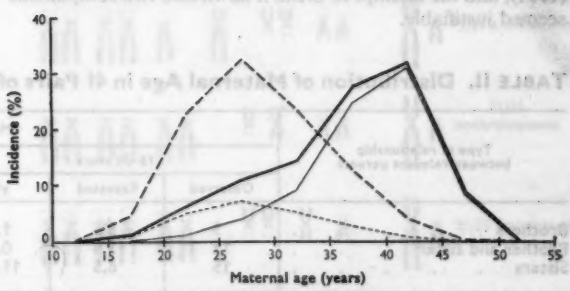
Few figures of incidence are available from non-European populations, although many cases have been reported among Africans, Indians, Japanese and Chinese. The general impression now is that the condition is less common outside Europe, but this is uncertain. It is at least clear from ethnographic study that there is nothing specifically "Mongolian" about these patients; indeed, on the basis of frequency, it might be more reasonable to use the name "European" to describe them. In the USSR the term "Down's disease" is used, and it might be preferable for everyone to use this or an equivalent eponymous title like "the Langdon-Down anomaly".

3. Relation to Maternal Age

Shuttleworth (1895) observed that nearly half his mongol patients were the last born of a long family. The relative significance of father's age, mother's age and order of birth remained obscure for a long time, since all three variables are mutually intercorrelated. The absence of paternal-age influence was first demonstrated (Jenkins, 1933; Penrose, 1933). Later, the more troublesome statistical separation of the effects of the order of birth from those of maternal age showed the mother's age alone to be significant (Penrose, 1934). It is not entirely ruled out that, exceptionally, the order of the birth, or even the father's age, might be a causal factor; indeed, Smith & Record (1955) consider that primogeniture is significant in this respect.

The distribution by maternal age, of births resulting in mongolism, compared with that in the general population for England and Wales, is shown in fig. 2. Essentially similar characteristics are present in all large sample records. Up till the age of 29 the distribution curve rises not appreciably more rapidly than the distribution curve for all births in the population. The incidence in this region is just about 1:2,000 births. After this point the distribution for all births falls steadily, though that for mongols continues to rise until after the maternal age of 40 years. The relative incidence for mongolism continues to rise even after the peak of absolute incidence has been recorded. An extensive inquiry into the offspring of mothers aged 45 years or more in London maternity hospitals, by T. Cohen and B. J. Warland (personal communication,

FIG. 2. Distribution of Births by Maternal Age



Mongols (M, see Table I)
All births in England and Wales, 1939 (A, see Table I)
Mongols (age-independent class, 22%)
Mongols (age-dependent class, 78%)

TABLE I. Distribution of Births by Maternal Age

Maternal age in years at birth of children	Percentage distributions		Partition of M		Percentage distribution	Relative incidence	Five-yearly fourfold increase in risk calculated for comparison with $\frac{B}{A}$
	1,038 cases of mongolism M	All births in population* A	Age-independent mongolism† (0.22 A)	Age-dependent mongolism‡ (0.78 B)	Age-dependent mongolism B	$\frac{B}{A}$	
15–19	1.0	4.6	1.0	0.0	0.0	0.00	0.01
20–24	6.1	22.6	5.0	1.1	1.4	0.06	0.04
25–29	10.8	32.3	7.1	3.7	4.7	0.15	0.15
30–34	14.1	23.4	5.1	9.0	11.5	0.49	0.58
35–39	27.3	12.7	2.8	24.5	31.4	2.47	2.31
40–44	31.9	4.0	0.9	31.0	39.7	9.92	9.23
45–49	8.8	0.4	0.1	8.7	11.1	27.75	36.92
All ages	100.0	100.0	22.0	78.0	100.0	1.00	1.00
Mean age	36.6	28.5	28.5	38.9	38.9	—	—

* England and Wales, 1939

† This is the maximal quantity of general population distribution which can be contained within the percentage distribution of 1,038 cases of mongolism (M).

‡ This column is obtained by subtraction of 0.22 A from M, in each age-group.

1960), revealed ten cases of mongolism among a total of 543 births, a relative frequency of 1:54—a value rather lower than that found in most previous surveys.

In view of the fact that, in some samples, notably those confined to first-born affected children, curves showing distributions of mongolism according to maternal age are bimodal, it has seemed probable that there are essentially two components and that the shape of the curve is determined by the proportions of two classes (see Table I). One of them, class A, could contain those cases in which maternal age was irrelevant and in which, consequently, it was distributed as for other births in the general population. In class B, the segment left after the removal of the largest possible age-independent class, the relative incidence of mongolism rises smoothly and almost exponentially with maternal age, perhaps increasing fourfold every five years. In fig. 2, the estimated proportions of class A and class B are 22% and 78%, respectively, but in more recently collected samples the proportion of class A is higher. Many observers have supposed the peculiar shape of this distribution curve to be an artifact of some kind, but the significance of its bi-tangentiality was confirmed by Haldane (1951), and the attempt to break it down into two components seemed justifiable.

4. Familial Concentration

It has, in the past, frequently been asserted that mongolism arises without any hereditary predisposition. Familial concentrations of cases are occasionally found but, with such a relatively common abnormality, it has been difficult to prove convincingly that such coincidences exceed random expectation. This is especially so in sibships, for the statistical risk of a second affected child is little more than double that expected in the population, taking maternal age into consideration. Analysis of collateral relationships, however, gave more convincing answers. For example, it was found that, on the average, when a patient's maternal relative was affected the mother's age at his birth was significantly lower than when a paternal relative was found to be affected (Penrose, 1951). The observed data concerning first cousins are particularly informative in this respect, as is shown in Table II. They are derived from published and unpublished material collected gradually over a period of 30 years. The expected numbers are based upon the maternal-age distribution of the control sample of 1,038 cases and upon the assumption that all four types of relationship, brother and brother, brother and sister, sister and brother, sister and sister, between parents of the two

TABLE II. Distribution of Maternal Age in 41 Pairs of First Cousin Mongols Classified by Type of Relationship

Type of relationship between relevant parents	Maternal age-group						Totals	
	15–34 years			35–49 years				
	Observed	Expected	χ^2	Observed	Expected	χ^2	Observed	Expected
Brothers	3	6.5	1.9	11	14.0	0.6	14	20.5
Brother and sister	12	13.0	0.1	28	28.0	0.0	40	41.0
Sisters	15	6.5	11.1	13	14.0	0.1	28	20.5
All types*	30	26.0	—	52	56.0	—	82	82.0

* The expectations are based on the control sample of 1,038 mongols in which 332 were born to mothers less than 35 years of age.

TABLE III. Offspring of Mongol Females

Description of child	Age of proposita at birth of her child	Maternal age at birth of proposita	Source
Normal female	25	?	Sawyer (1949)
Mongol male .	30	42	Lelong, Borniche, Kreisler & Baudy (1949)
Mongol female.	19	30	Rehn & Thomas (1957)
Retarded male.	30	42	Forssman & Thysell (1957)
Idiot female .	29	39	Schlaug (1957)
Mongol female.	21	44	Hanhart (1960)
Mongol male .	23	44	Hanhart (1960)
Normal male .	?	?	Levan & Hsu (1960)

first cousin mongols are equally likely. The total χ^2 value of 13.8 for 5 degrees of freedom owes its significance entirely to the young mothers, whose sisters also have mongol children, for which χ^2 is 11.1.

Further striking evidence concerning hereditary influences in mongolism came from accounts of the result of pregnancies in affected females. Table III summarizes the published material. Altogether, of the eight recorded offspring, four are certainly mongols, all born at comparatively young maternal ages of 30, 19, 21 and 23 years, respectively.

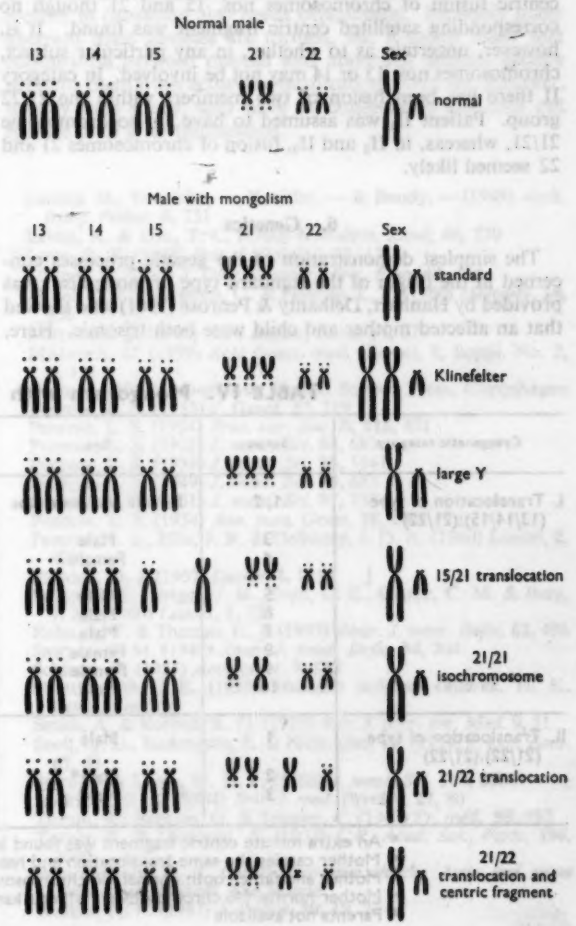
An additional line of inquiry about hereditary disposition concerns the examination of normal close relatives for characteristic minor signs of mongolism. With qualitative traits, like presence of transverse palmar creases and fissured tongue, Turpin & Caratzali (1933) obtained positive results. Some metrical traits, like the number of dermal ridges (Fang, 1950), or the degrees of angles between triradii[1], can be precise statistically. Investigation of the position of the palmar triradius *t*, from this point of view, led to the conclusion that mothers and, to some extent, brothers and sisters, but not fathers, had slight but significant resemblance to the affected child (Penrose, 1954). A survey of blood groups in cases of mongolism (Lang-Brown, Lawler & Penrose, 1953) revealed no indication that antigenic incompatibility could have a causal influence; rather there was increased similarity of mother and affected child.

5. Cytology

It has long been suspected that some unusual genetic mechanism might be responsible for mongolism and, by analogy with plants and experimental animals, a specific chromosome aberration or lack of chromatin balance seemed indicated. Waardenburg (1932) expressed this view clearly and considered non-disjunction to be the most likely cause. One difficulty in accepting this suggestion was the absence of experimental evidence showing that such a process could be influenced by maternal age. The familial examples, moreover, implied another mechanism, in particular some type of translocation, as a likely cause (Penrose, 1939), and Snell, Bodemann & Hollander (1934) had actually demonstrated malformation initiated by such aberrations in mice. Attempts to examine the chromosomes in mongolism directly (Mittwoch, 1952) led to the conclusion that, in diakinesis, there were 24 chromosomal masses; this was, at that time, the accepted normal number, not 23, as shown later by Ford & Hamerton (1956). It was not until Lejeune, Gauthier & Turpin (1959) analysed the chromosomes in cultures from connective tissue that the reality of some early conjectures was demonstrated. These investigators were able to show that a specific lack of chromatin balance was present, caused by the triplication of a small acrocentric chromosome generally assumed to be no. 21 (Denver system). It is natural to suppose that this aberration is the result of non-disjunction during gametogenesis, and the proved association with maternal age points to the ovum rather than the sperm as the point of origin.

A number of variants of the mongol karyotype have also been described and analysed (see fig. 3). First, the association with Klinefelter's syndrome (Ford, Jones, Miller, Mittwoch, Penrose, Ridler & Shapiro, 1959) produced the characteristics of both conditions superimposed; the same set of 48 chromosomes was eventually demonstrated in cultured cells from three tissues—bone-marrow, skin and peripheral blood. Another variant, thought probable by Jacobs, Baikie, Brown

FIG. 3. Human Karyotypes Showing Acrocentric and Sex Chromosomes Only



[1] See Holt, p. 247 of this number of the Bulletin.—Ed.

& Strong (1959) and noted since by several observers (e.g., J. H. Edwards and G. Clarke, personal communication, 1960), is the incomplete or mosaic type in which a proportion of cells, from 10% to 50%, are normal with 46 chromosomes. Such a patient would theoretically be rather mildly affected, and the question is raised whether or not some quite normal people with abortive signs of mongolism may not have a minority of trisomic cells. In mongolism associated with leukaemia, the standard karyotype with 47 chromosomes has been found in blood and in other tissues (A. G. Baikie, personal communication, 1960). Recently a male mongol with an unusually large Y chromosome has been described by P. A. Jacobs and D. G. Harnden (personal communication, 1961).

Examples of chromosome translocations are not infrequently found in clinically typical mongols; these affect the two groups of chromosomes nos. 13, 14, 15 and 21, 22, all of which are acrocentric and satellited. The clinical result is practically the same as in typical mongols trisomic for chromosome 21, although there are only 46 chromosomes in the karyotype, because this unbalanced state is very nearly equivalent to that produced in the standard trisomic condition. A list of cases of translocation, known at the time of writing, is given in Table IV. In category I, most examples appeared to show a centric fusion of chromosomes nos. 15 and 21 though no corresponding satellited centric fragment was found. It is, however, uncertain as to whether, in any particular subject, chromosomes nos. 13 or 14 may not be involved. In category II there has been fusion of two members within the 21/22 group. Patient II_1 was assumed to have an isochromosome 21/21, whereas, in II_2 and II_3, fusion of chromosomes 21 and 22 seemed likely.

6. Genetics

The simplest demonstration of the genetic processes concerned in the origin of the standard type of mongolism was provided by Hanhart, Delhanty & Penrose (1961) who showed that an affected mother and child were both trisomic. Here, primary non-disjunction must have caused the disease in the mother and secondary non-disjunction in the child. In the family described by Rehn & Thomas (1957) the affected child was shown to be trisomic (A. W. Johnston, personal communication, 1961), but the mother was not available for testing. Secondary non-disjunction is a direct consequence of abnormal meiosis in the trisomic parent, but is not observable in the female. However, in testicular material from a mongol, Miller, Mittwoch & Penrose (1960) interpreted some cells in diakinesis as showing 22 bivalents and one trivalent and others as showing 23 bivalents and one univalent.

In examples I_1 to I_7 (Table IV) all the parents were tested and the normal mothers were found to carry the same fusion and to have only 45 chromosomes. Though it may be presumed that a centric fragment has been lost following translocation, they have almost the normal amount of chromatin. Evidently they can produce balanced gametes, normal or translocated, and also unbalanced gametes. In the family of patients I_1 and I_2 the fused chromosome was demonstrated in three generations of normal females, the mother's mother, the mother and the sister of the patients. It is noteworthy that in no instance, so far, has the father of a mongol been found to have this peculiarity; patient I_7 had a normal brother who showed this fusion: he had two children dying in infancy, but they were not thought to have been mongols. The maternal ages, for all these mongols, were less than 34 years, and it is evident that, in mothers predisposed by carrying such a translocation, the induction of mongolism is independent of age. Patient I_8 is a member of a pedigree (Penrose, 1938) used to demonstrate a hereditary factor in mongolism transmitted through related females.

More puzzling are the instances where the same type of translocation has appeared in a mongol whose parents have normal karyotypes, as in cases I_8, I_9 and I_{10}. In case I_9, moreover, the patient has two normal brothers (46 chromosomes each) and a mongol sister with standard karyotype of 47. The ages of the mothers of these affected children are all low, and the hypothesis of maternal gonadal mosaicism seems

TABLE IV. Mongolism with Translocation (46 Chromosomes)

Cytogenetic category	Series	Sex	Maternal age (years)	Source
I. Translocation of type (13/14/15):(21/22)	1, 2	Female and male sibs	23, 28[a]	Penrose, Ellis & Delhanty (1960)
	3	Male	23[a]	Carter, Hamerton, Polani, Gunalp & Weller (1960)
	4	Female	31[a]	J. H. Edwards and G. Clarke, personal communication (1960)
	5	Male	24[a]	J. D. A. Delhanty and L. S. Penrose, unpublished (1961)
	6	Male	32[a]	Buckton, Harnden, Baikie & Woods (1961)
	7	Male	28[a]	O. J. Miller, personal communication (1960)
	8	Female	19[b]	Polani, Briggs, Ford, Clarke & Berg (1960)
	9	Female	31[b]	Penrose and Ellis, unpublished (1961)
	10	Male	26[b]	Ellis, personal communication (1960)
II. Translocation of type (21/22):(21/22)	1	Male	39[c]	Fraccaro, Kaijser & Lindsten (1960)
	2	Male*	33[d]	Penrose *et al.* (1960)
	3	Male	39[d]	Bavin and Marshall, personal communication (1960)

* An extra minute centric fragment was found in some cells
[a] Mother carries the same translocation and has 45 chromosomes; father normal (46 chromosomes)
[b] Mother and father both normal (46 chromosomes)
[c] Mother normal (46 chromosomes); father's karyotype abnormal (47 chromosomes)
[d] Parents not available

attractive. In category II less is known about genetics though, eventually, paternal inheritance may be found important. The parents of patient II_1 were tested and the father was shown to carry an unidentified extra chromosome; unfortunately, the parents of patients II_2 and II_3 were not available for study.

It must be emphasized that only a minority of examples of familial mongolism or of mongols born to young mothers have translocations. By analogy with *Drosophila*, special genes can produce non-disjunction in the ova of homozygous females. In human pedigrees, evidence for this could be sought by noting consanguinity in parents of mothers of trisomics. In a series of over 600 mongols, collected during 30 years, only three with parents who were cousins were found, but five of the fathers and ten of the mothers had cousin parents. Though the comparatively large figure for mothers' parents is suggestive, it is of quite insignificant dimensions. However, the coincidence of two kinds of trisomy, as in Klinefelter mongolism, which is perhaps commoner than originally supposed, may be evidence of a general hereditary tendency to non-disjunction in one parent.

7. Conclusion

It is now possible to summarize what is known of the causation of mongolism. Class A, where the occurrence is independent of maternal age, includes several different deviations from normal chromosomal mechanisms which can be listed as follows:

i. secondary non-disjunction directly caused by trisomy of chromosome no. 21 in the mother (very rare);
ii. errors in meiosis caused by a balanced translocation, in the mother, involving chromosome no. 21;
iii. errors of types (i) or (ii) proceeding from gonadal mosaicism in the mother;
iv. primary non-disjunction facilitated by maternal genetic constitution or by unidentified environmental influences.

In class B, a process very closely related to the age of the mother or to the age of the ovum itself is implicated. In view of the curious affinity of the satellited chromosomes for one another, observable in mitosis (Harnden, 1961), and their general similarities in structure, it is difficult not to infer that there is a natural risk of aberration among them. The translocation types observed, moreover, are all similar in that they appear to be centric fusions not caused by random breakages and subsequent rearrangements. The most obvious explanation is that there is some degree of homology between the acrocentric chromosomes, i.e., they are homologous. Whether the ordinary non-disjunction associated with maternal age is brought about by dehydration, hormonal action, infection, or by some quite different process which might be determined by genes, is still entirely obscure. The predominance of the mother in causation also needs explanation. Probably many aberrant male gametes are formed, but it seems likely that competition between normal and abnormal sperms usually prevents them from achieving fertilization. Ova are, however, usually shed singly, they have no competitors and may not mature until after fertilization.

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INHERITED VARIATIONS IN LEUCOCYTES

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1 Dominant variations
a Anomalies of the nucleus
b Anomalies of the cytoplasm
2 Recessive variations
References

Although the existence of the leucocytes has been known for almost two centuries (Hewson, 1777) and they have been studied in detail for over 70 years since Ehrlich (1891) introduced his aniline stains, it is only in relatively recent times that inherited variations in their structure have been recognized. Many of these variations are closely linked with the steps in the development of the leucocytes through the animal kingdom, and their importance is becoming more apparent as it is realized that they can be used as natural experiments to examine the functions of the microscopic structures in the cells. They also provide excellent "markers" for genetic and other studies.

The number of recognized inherited variations in leucocytes has been growing steadily, and now at least ten distinct types are known and many hundreds of families have been investigated. Some are inherited as dominants and are apparent in the heterozygous state; others are recessive and are found only in individuals who are homozygous for the appropriate gene, which they must have inherited from both, frequently consanguineous, parents. The known variations are listed in Table I.

TABLE I. Known Variations in Leucocytes

Part of cell affected	Anomaly	Variants	Reference
	DOMINANT INHERITANCE		
Nucleus	Pelger-Huët neutrophil anomaly	—	Pelger (1928)
	i. Heterozygous form	—	Huët (1931, 1932)
		Trisegmented nuclei present	Ebbing (1959)
		Atypical nuclei	Ebbing (1959)
		Rounded "Stodtmeister" nuclei	Undritz (1954)
		High and low frequency types	Undritz (1937)
	ii. "Partial form"	—	Begemann & Campagne (1952)
	iii. Homozygous form		
	Hereditary constitutional neutrophil nuclear hypersegmentation	—	Undritz (1939)
	Hereditary constitutional eosinophil nuclear medium hypersegmentation	—	Undritz (1943, 1954)
	Hereditary prevalence of nuclear appendages ("caryoschises")	—	Seman (1959)
	Hereditary giant neutrophils	—	Davidson, Milner & Lawler (1960)
Cytoplasm	May–Hegglin anomaly	—	May (1909); Hegglin (1945)
	Hereditary lymphocytic vacuolation	—	von Bagh & Hortling (1948)
	RECESSIVE INHERITANCE		
Cytoplasm	Alder–Reilly granulation anomaly	—	Alder (1939); Reilly (1941)
	i. Homozygous form	Incomplete types: (a) Neutrophils affected (Hottinger–Eichenberger type) (b) Lymphocytes affected (Gasser–Zellweger type) (c) Monocytes affected (Grgić–Kalafatić type)	
	ii. Partial form where only some neutrophils are affected	—	Alder (1939)
	Constitutional granular giantism	—	Béguez César (1943); Chediak (1952)
	i. Homozygous form with albinism ii. Homozygous form without albinism	—	Steinbrinck (1948)
	Other anomalies of the granules	—	Mittwoch (1959); for discussion see Alder (1939); Reilly (1941)
	Peroxidase defect in the neutrophils	—	Alius, cited by Undritz (unpublished)
	Hereditary constitutional agranulocytosis	—	Kostmann (1957)

1. Dominant Variations

a. Anomalies of the Nucleus

Pelger-Huët Anomaly

This is an inherited defect in the process that normally leads to lobulation of the nuclei in the neutrophils and eosinophils. As a result, few, if any, of the mature neutrophils, and none of the eosinophils, have more than two lobes in their nuclei. The anomaly was discovered by Pelger (1928) who considered it to be an unusual reaction to tuberculosis. Huët (1931, 1932) found the same phenomenon in relatives of one of Pelger's patients and established its hereditary nature.

The anomaly is relatively common and persists throughout life (Undritz & de Sepibus, 1957). It was found approximately once in every 6,000 routine blood films examined in a general hospital in London (Davidson, Lawler & Ackerley, 1954), and was even more frequent in Switzerland (Undritz, 1939). Over 150 families so affected have been reported from all parts of the world. Cells characteristic of this anomaly are illustrated in Plate I, A.

The anomaly is manifest in subjects heterozygous for the gene, and with only doubtful exceptions it has been inherited as a simple dominant (see Undritz, 1944, 1959) affecting half the members of a sibship.

Only in one instance has the homozygous state of the Pelger-Huët anomaly been established fully by showing that both parents were heterozygous (Begemann & Campagne, 1952) (Table II; and Plate I, Ap, Aq). Three other examples have been recorded (Bernard, Undritz, Bru, Mathé & Toulouse, 1956; Ciplea & Ciorapciu, 1958; Stobbe, 1959a); but, without adequate family studies, it is possible that the rounded nuclei arose as the result of a shift to the left in a heterozygous or perhaps even in a normal subject (see Baltzer, 1953) (Table II; and Plate I, B).

In individuals who are heterozygous for the gene determining the Pelger-Huët anomaly, the vast majority of the neutrophils, although mature in every other way, have either unsegmented, dumb-bell shaped, or bilobed spectacle-like nuclei (Plate I, Aa, Ab, Ac). Only a few of the neutrophils have three-lobed nuclei, except in the rare instances described as a family feature by Ebbing (1959). Usually, round nuclei are confined to the marrow precursors and only rarely is one found in a mature neutrophil (Plate I, An), but almost round, pomegranate-shaped nuclei, the "Stodtmeister type", were a feature of the blood in a family found by Birk and described by Petzel & Undritz (1957) (Plate I, Ah, Am).

When Undritz (1937, 1947) reported a "partial form" (*Teilträger*) of the anomaly in which the affected individuals had only 5–20% unlobed or bilobed neutrophils of the Pelger-Huët type (Table II; and Plate I, Ag), some doubt was felt, as such cells are to be found even in normal blood films (Plate I, Ad). Recently, however, confirmation of the existence of a partial form, having a close affinity with the true Pelger-Huët phenomenon, has come from finding families in which a proportion of the neutrophils have nuclei of the more strikingly abnormal "Stodtmeister" or "flea-like" types (Petzel & Undritz, 1957) (Table II). The genetic relationship between the partial and true forms has not been defined, but there is one report, as yet unconfirmed, of parents with the partial form having a child with the fully developed Pelger-Huët anomaly (R. Stahel, cited by Undritz, 1944). It has been suggested that the partial form may be the result of a mosaicism, the marrow producing two separate lines of normal and Pelger-Huët leucocytes. The phenomenon, however, must have a different origin from the neutrophil mixture in the blood chimaeras (Davidson, Fowler & Smith, 1958).

TABLE II. Typical or Composite Examples of the Variations in Nuclear Structure

(The numbers of Pelger-like cells are given in italic numerals.)

Types of anomaly	Nuclear structure... (percentage of degrees of lobulation of nuclei)						
	Round	Unlobed	Lobed				
			2	3	4	5	6
Neutrophils							
Normal	—	4	26	48	19	3	—
Shift to left	—	16	45	35	4	—	—
Shift to right	—	—	1	14	38	30	17
Pelger-Huët anomaly							
Heterozygote	—	42	*55*	*3*	—	—	—
Atypical	—	*51*	*41*	*7*	*1*	—	—
"Stodtmeister"	*26*	*73*	*1*	—	—	—	—
Homozygote	*90*	*10*	—	—	—	—	—
Partial form (Undritz)							
High frequency	—	*8, 7*	*11, 16*	32	22	4	—
Low frequency	—	4	*1, 20*	52	20	3	—
"Stodtmeister"	*13*	*29, 5*	*8, 12*	*1, 19*	11	2	—
Pseudo-Pelger change							
Heterozygous type	—	2	80	17	1	—	—
Atypical	—	70	27	3	—	—	—
Homozygous type	52	43	3	2	—	—	—
Hypersegmentation							
Heterozygote	—	—	6	30	44	17	3
Homozygote	—	—	2	15	61	16	6
Eosinophils							
Normal	—	3	68	27	2	—	—
Hypersegmentation	—	—	9	55	32	4	—

Such pseudo-Pelger changes can occur in chronic infections, particularly those affecting the bowel, in glandular fever, in malaria, in leukaemia, and as a result of bony metastasis (Dorr & Moloney, 1959). They can also be induced by toxic agents and drugs, particularly colchicine (Harm, 1953), and may appear in stored blood (Plate I, Bd). Even a homozygous-like change can be acquired (Lüers, Nachtsheim & Petzel, 1956), especially in leukaemia (Darte, Dacie & McSorley, 1954) (Plate I, Bc).

In individuals with the Pelger anomaly the nuclear change present in pus cells is often exaggerated by a left shift (Plate I, Ah, Ak). The nuclear structure in the Pelger-like change differs from that of the true homozygous Pelger type in being less shrunken and clumped (Plate I, Am, Aq, Bc), and Stobbe (1959a) has suggested that this indicates a difference in the intranuclear protein in the Pelger anomaly.

Although not demonstrable, some change may be present in the nuclei of the tissue cells but, while few sex "drumsticks" can be demonstrated in the neutrophils (Lüers & Petzel, 1958; Davidson & Smith, 1960) (Plate I, Af), the frequency of their sex chromatin is normal. The Pelger neutrophils, even in the homozygous form, function normally as phagocytes (Davidson *et al.* 1954; Stobbe, 1959b).

No association or linkage has been established between the heterozygous state and predisposition to any disease or deformity, nor has any been shown between the Pelger anomaly and the blood-group systems (Davidson *et al.* 1954, and unpublished), and the affected subjects are healthy and may live to a great age.

Leucocytes with bilobed nuclei are a normal feature in many animals (Undritz, 1943), and the Pelger anomaly has been demonstrated in rabbits and toads (Undritz, 1944). The heterozygous rabbits appear to be completely normal even in their reaction to immune haemolysins (Davidson, unpublished), but the mating of heterozygotes results in small litters. Only Nachtsheim has succeeded in rearing a homozygote to sexual maturity (Undritz, 1944), and it was small and had skeletal deformities. Direct examination of the chromosomes in rabbits (Davidson and Norman, unpublished) and in man (Stobbe, 1959a) has failed to establish any morphological change.

The Pelger change is of use as a marker of heredity, and as a means to follow the survival of transfused leucocytes (Rosse & Gurney, 1959) or of grafted marrow cells after total body irradiation (Plate I, Be, Bf) (Davidson, unpublished).

Hereditary Constitutional Hypersegmentation of the Neutrophil Nuclei

Hypersegmentation of the neutrophil nuclei has long been known as an acquired change in pernicious anaemia and chronic iron deficiency anaemia; as a hereditary anomaly it was first described in rabbits, and later in man, by Undritz (1939). In normal human blood the majority of the cells have three-lobed nuclei, but in hypersegmentation, otherwise known as a shift to the right or senility of the neutrophils, the average number of the lobes may rise to over four. Similarly, in two families described by Undritz (1958d) the majority of the cells had four or more lobes (Table II; and Plate II, Ca). This appeared to be inherited as a simple dominant and not to have any adverse effect. In the second family, Undritz found a probable homozygote, with an even more marked shift to the right.

The normal size of the neutrophils has been taken as evidence against the condition's being due to tetraploidy (Undritz, 1958d). Likewise, although Lüers (1960) found an increased frequency of drumsticks (69 instead of 28 per 1,000), she did not find any neutrophils with two drumsticks.

Hereditary Constitutional Hypersegmentation of the Eosinophil Nuclei

This condition, described by Undritz (1954), corresponds to the previous anomaly, but affects the eosinophils. Normally two-thirds of the eosinophils have bilobed nuclei, and most of the others have three lobes, with only small numbers of unsegmented or four-lobed cells (Table II; and see Plate II, Cb). A composite picture of the lobing of 8,763 eosinophils from over a hundred nurses and female patients is given in Table II. In an otherwise normal mother and daughter, Undritz (1944, 1954) found many four-segmented, or even five-segmented, eosinophils, and I discovered an isolated instance of a similar distribution while preparing the composite picture (Table II).

Hereditary Prevalence of Nuclear Appendages

Since the description by Davidson & Smith (1954) of a sex difference in the neutrophil leucocytes it has been known that, in contrast to the drumsticks, thread-like and other small projections are commoner in the male; but these are not sex specific, for they may be met in the nuclei of some females (Davidson & Smith, 1960). Gruner (1915–16) suggested a relationship between a high frequency of nuclear appendages and carcinoma. In 1959 Seman described a family in which this feature appeared to be an inherited characteristic. Such appendages were present in 76% of the neutrophils in the propositus and in 25–56% in his father, uncle, two sons and a daughter, but in neither of his wives. The description would be more convincing if an adequate control series had been presented.

The great variability in the frequency of the sex drumsticks, even in normal females, is probably related to this condition. The extremes lie between 1 in 10 and 1 in 150, or even in 200, quite apart from any variation due to alteration in the lobing of the nucleus (Davidson & Smith, 1958). Although groups of individuals who show a shift to the left have few drumsticks, and the frequency is increased in groups with a shift to the right, many exceptions occur and it has been suggested, but not proved, that there is a hereditary influence (Davidson & Smith, 1958).

A curious finding has been that in about half the cases of chromatin-positive Klinefelter's syndrome (a condition due to extra sex chromosomes, probably arising from a primary

PLATE I. INHERITED VARIATIONS IN LEUCOCYTES

FIG. A. The Pelger-Huët anomaly

(Illustrations are lettered a to q, in columns from above downwards.)

a: two typical neutrophils from the heterozygous form; b: three typical neutrophils from the heterozygous form; c: neutrophil and eosinophil leucocytes from the heterozygous form; d: an isolated "Pelger-like" cell from a normal blood film; e: a Pelger neutrophil and eosinophil with a further "shift to the left" in tuberculosis; f: rare occurrence of a typical female "drumstick" in a Pelger cell; g: one of the bilobed neutrophils and a normal segmented neutrophil from a 20% partial form (Undritz); h: a "Stodtmeister" neutrophil with its typical rounded nucleus and group of projections from a 50% partial form; j: marrow with many small myelocytes; k: pus cells; l: sputum cells (same case as in fig. Ae), from the heterozygous form; m: "partial carrier" with 20%, nearly round, Stodtmeister cells; n: a nearly round nucleus rarely found in the heterozygous form; p: the typical round nuclei with coarse chromatin clumps in two neutrophils; q: an eosinophil from the homozygous form

FIG. B. The pseudo-Pelger change as a manifestation of a "left shift" or of a degeneration

a, b, c: neutrophil leucocytes in ulcerative colitis; in glandular fever; in chronic myelogenous leukaemia; d: a degenerate neutrophil in stored oxalated blood

The artificial Pelger change

e: the neutrophils, showing female drumsticks, from a normal rabbit; f: cells from the marrow of the same rabbit, showing Pelger characteristics, following whole-body irradiation and colonization by grafted marrow cells from a donor with Pelger anomaly

INHERITED VARIATIONS IN LEUCOCYTES

(FIGS. A–E)

William M. Davidson

(page 190)

FIG. A

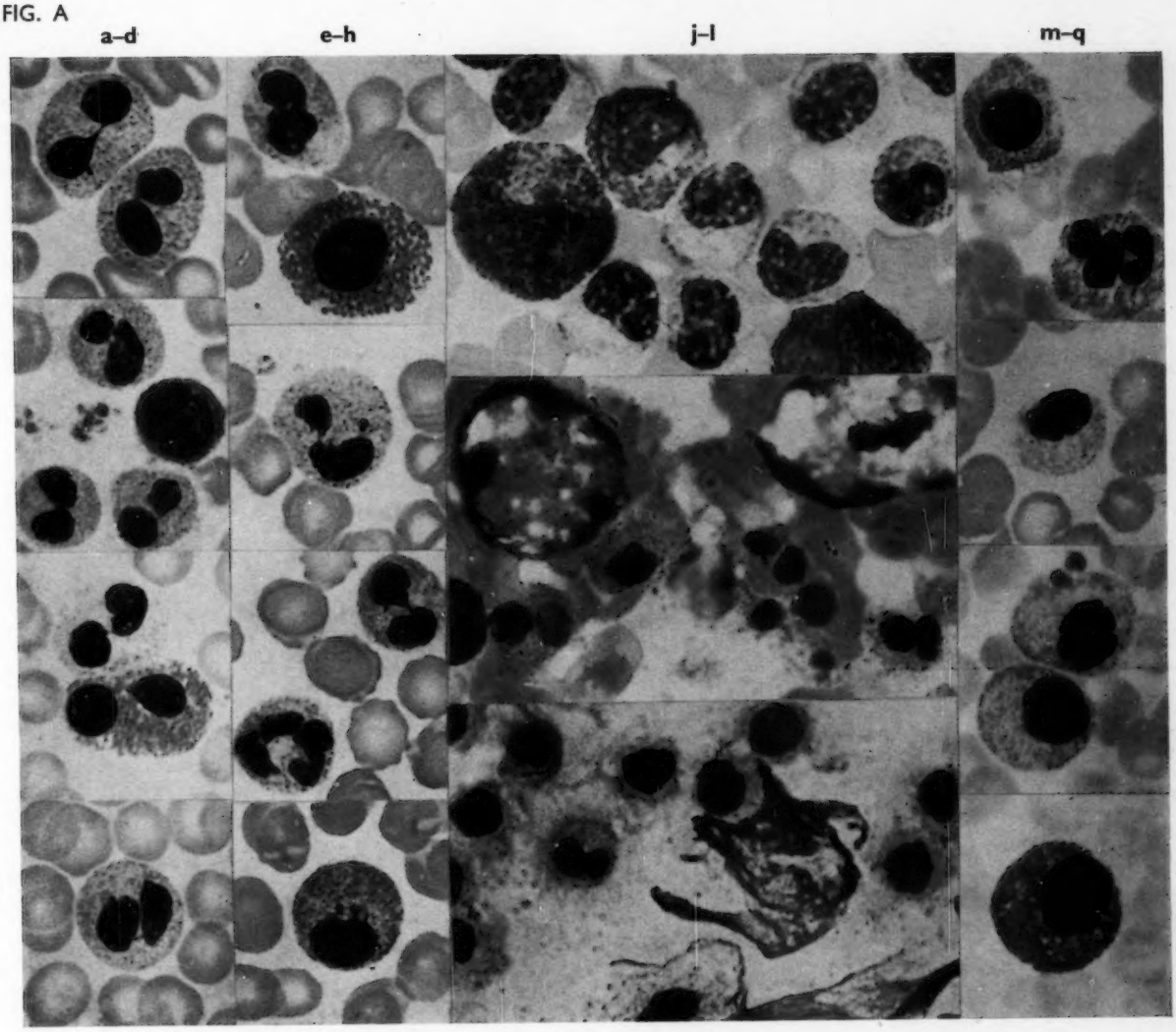

FIG. B

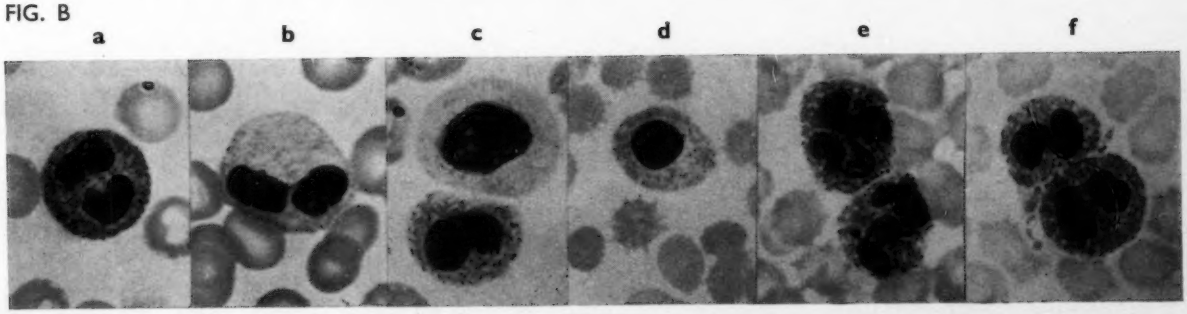

FIG. C

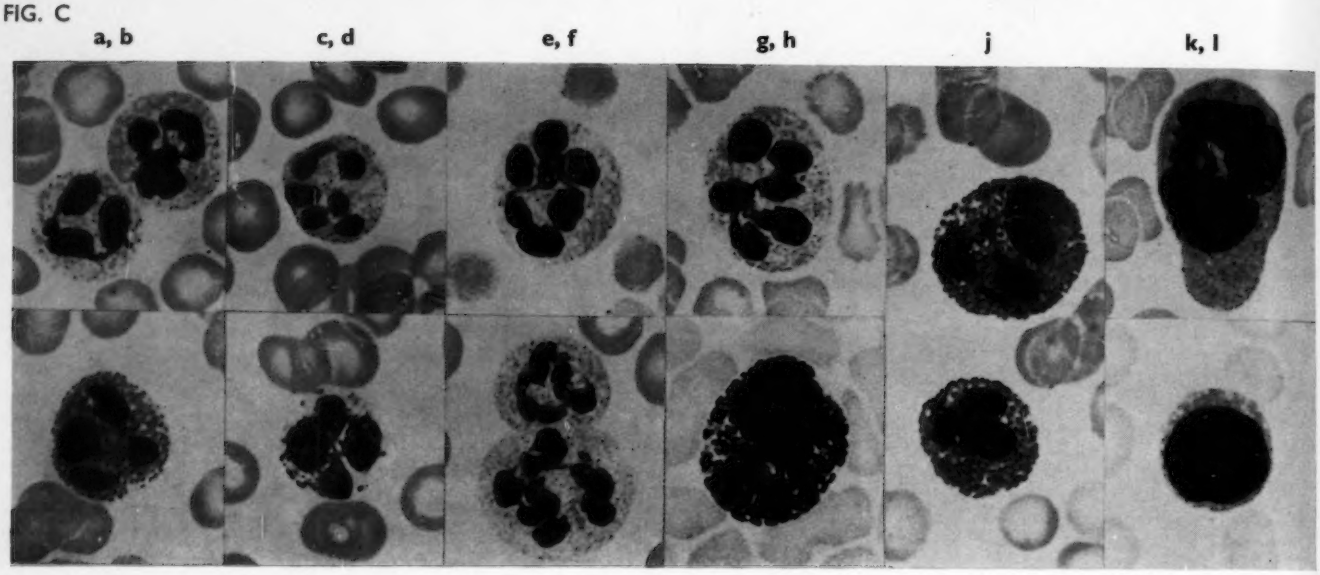

FIG. D

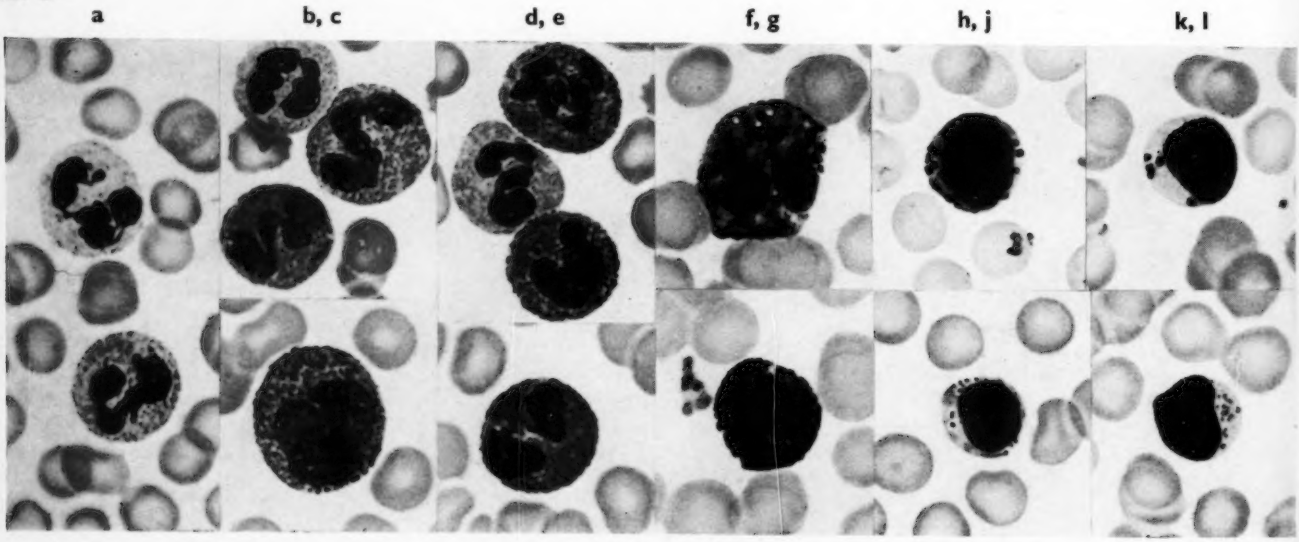

FIG. E

non-disjunction, possibly hereditarily determined) the frequency of the drumsticks is low despite the fact that there are adequate numbers of sex nodules in the tissues (Davidson & Smith, 1958). The possibility of this being due to a mosaic state has been considered. There is also the possibility that in mongolism the additional small chromosome is a factor in the observed nuclear shift to the left in neutrophils (Turpin & Bernyer, 1947; Mittwoch, 1958).

Hereditary Giant Neutrophil Leucocytes

Isolated giant leucocytes, with an average diameter of about 17 μ, are met with not infrequently in blood films from patients, but are very much less common in the healthy (Plate II, C), and certainly never exceed 2 per 1,000 neutrophils (Davidson *et al.* 1960). Usually their presence is associated with a severe toxic upset to the marrow, and a very high count (38 per 1,000) has been found during treatment with triethylenemelamine for carcinomatosis. In 1955 a family was discovered in which giant neutrophils appeared to be inherited as an anomaly (Davidson *et al.* 1960). The propositus had 16 per 1,000 neutrophils, and his mother, grandfather, grandaunt, and two uncles were affected in a similar way. All were in good health and it was shown that the condition persisted for at least two years. No association could be demonstrated between inheritance and any disease process or deformity, any of the blood groups, the secretor state, the serum groups, or the haptoglobins.

So far only the heterozygous form has been encountered and it seems to be a harmless variant. Certainly no gross metabolic upset could be demonstrated and at least two affected individuals have reached 80 years of age.

b. Anomalies of the Cytoplasm

May-Hegglin Anomaly (Hereditary Polyphylic Disorder of Maturation)

This is not a very well-confirmed anomaly and may be simulated readily by changes due to disease. In 1945 Hegglin described a man and his two sons who, although in good health, had a combination of a mild leucopenia, with Döhle bodies (Döhle, 1912) in all their neutrophils (Plate II, Ed, Ee), and a slight thrombocytopenia with giant forms of platelets. Thirty-one near relatives were unaffected.

A rather similar condition had been described by May in 1909, in a girl who had anaemia following a haematemesis and possibly a disproportionately severe oedema of her legs. The "inclusion bodies" found in all her neutrophils persisted after she recovered. They were rod- or comma-shaped, stained a greenish-blue with the Romanowsky stains, and intensely red with pyronine, but contained neither glycogen nor lipid. This is in keeping with their having been Döhle bodies, which are 1–2 μ in length and formed of ribonucleic acid (Hansson, Linell, Nilsson, Söderhjelm & Undritz, 1959).

Recently Scholer (Scholer, Im Hof & Schnös, 1960) published another isolated case occurring in a healthy male. From extensive investigation of the clotting mechanism it was concluded that there was a qualitative platelet defect. A patient described by Leitner (Leitner, Neumark & Heeres, 1954) had an acute illness suggesting a septicaemia, and the case cannot be considered to be a genuine example of the May-Hegglin anomaly.

Döhle bodies were first described as an acquired phenomenon in scarlet fever (Döhle, 1912) and may be found in acute infectious and toxic processes, where they could be

PLATE II. INHERITED VARIATIONS IN LEUCOCYTES

FIG. C. Further inherited and acquired variations in the nuclei
(Illustrations are lettered in pairs above and below, a to l.)

Inherited hypersegmentation

a: anomalous hypersegmentation of neutrophil nuclei

Acquired hypersegmentation found in routine blood films

b: hypersegmentation of an eosinophil nucleus, an accidental finding; c: hypersegmentation of the neutrophil nucleus; d: hypersegmentation of the basophil nucleus

Inherited giant neutrophils

e: a giant neutrophil, one of many present in the blood of the propositus as an inherited anomaly

Acquired giant white cells from routine blood films

f: a normal neutrophil and a giant form showing a typical female drumstick; g: a giant neutrophil, one of many, the result of tetraethylenemelamine treatment for inoperable cancer of the lung; h: a giant basophil; j: a giant and a normal eosinophil; k: a giant monocyte; l: a giant lymphocyte

FIG. D. Nuclear appendages
(Illustrations are lettered in pairs above and below, a to l.)

a: two neutrophils, from a normal female, one showing a typical drumstick, the other many small threads and tags

Acquired and inherited variations in the cytoplasmic granules

b: toxic granulation of the neutrophil leucocytes in infection; c, d, e, f, g, h: the Alder-Reilly anomaly affecting the granules in a monocyte; two neutrophils, with between them a normal neutrophil in a double blood smear; an eosinophil showing only a minor change; an eosinophil from another family, showing a marked change; rather heavy Alder-Reilly granules in a basophil; and prominent granules in a lymphocyte; j: rather similar granules and vacuoles in a lymphocyte in "gargoylism"; k, l: the most extreme granules found in normal lymphocytes, for comparison with figs. Dh and Dj

FIG. E. Cytoplasmic vacuolation

a: large vacuoles in a lymphocyte in amaurotic family idiocy; b: large and small vacuoles in amaurotic family idiocy; c: vacuolated blast cells in acute leukaemia; d, e: Döhle bodies in neutrophils in infection; f: the coarse clumped granules in the neutrophils in the Chediak anomaly (from Hansson *et al.* 1959, by permission of the editor)

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I am indebted for the blood films from which a number of the anomalies have been photographed to Dr Eric Undritz (figs. Ag, Ah, Am, Ap, Aq; Ca; Dh); Professor H. R. Wiedemann (figs. Df, Dg); Dr E. Neumark (figs. Ed, Ee); and Dr Ursula Mittwoch (fig. Dj). I am also grateful to Mr S. Pegg, Senior Technician in my Department, for his help in preparing the photographs.

associated with a thrombocytopenia or a capillary defect. They have been extensively investigated in relation to burns by Weiner & Topley (1955).

Hereditary Vacuolization of the Lymphocytes

Small definite vacuoles are not uncommonly associated with the granules in the cytoplasm of normal lymphocytes (Plate II, Dl), but large clear-cut vacuoles are abnormal (Plate II, Ea, Eb, Ec). Such a change was first described in amaurotic family idiocy by von Bagh & Hortling (1948) and has recently been found associated with another inherited disorder of lipid metabolism, Niemann-Pick's disease (Cumings and Goodbody, personal communication cited by Mittwoch, 1959; Sandifer, 1959). In amaurotic idiocy only some of the lymphocytes are affected and there appear to be two types of vacuolation. Well-defined vacuoles of up to 2 μ in diameter, 10 to 15 filling the cytoplasm, are typical of the homozygous state presenting as amaurotic idiocy, and a smaller rather ill-defined type may be a precursory form (Jelgersma, 1960). It has been suggested that the second type is also to be found in heterozygotes, and in Jelgersma's family they were demonstrated in two normal sibs, the father, and a paternal uncle and aunt, but not in the mother of the propositus. During a recent investigation they were not found in the mother, father or sibs of an amaurotic child whose lymphocytes showed typical vacuolation (Plate II, Ea, Eb). These findings cast doubt upon the dominant mode of inheritance and on the use of vacuolation as a method of making a diagnosis of amaurosis in the pre-clinical stage of the disorder (Mittwoch, 1959).

2. Recessive Variations

A range of anomalies, affecting especially the cytoplasmic granules, has been described.

Alder-Reilly Anomaly

This anomaly of the leucocytes, which makes the granules more prominent, was discovered independently by Alder (1939) and Reilly (1941). Alder described the abnormal leucocytes in a brother and sister who later at puberty developed changes in their hip joints (Alder, 1950). Reilly discovered the curious leucocyte granules in four of eight cases of "gargoylism" (Ellis, Sheldon & Capon, 1936), otherwise known as the Hurler-Pfaundler syndrome. This comprises a peculiar facies, skeletal changes, hepatosplenomegaly, corneal opacity and low intelligence. Later Reilly & Lindsay (1948) described the frequent association of the anomaly with anaemia and thrombocytopenia, and recognized that the condition was part of a metabolic disorder with abnormal polysaccharide storage.

In many of the 75 recorded cases of the anomaly (François, Barbier & Rouck, 1959) there were some or all of the features of gargoylism (Fricker-Alder, 1958), but other patients have had lesser changes or even lacked them altogether (Undritz, 1954). The anomaly is usually inherited as a recessive trait, but families have been described by Jordans (1947) and François *et al.* (1959) in which it was dominant.

At one time it was believed that the affected children did not live beyond ten years, but the boy originally described by Alder was in good health at 28 and the skeletal changes had not advanced (Fricker-Alder, 1958); a personal case was discovered at 65, and Undritz (1958a) mentions the anomaly as being found in a woman aged 80.

The prominent granules in the neutrophils take a deep lilac colour with the Romanowsky stains and in the eosinophils many stain violet or green as in the myelocytes (Plate II, Df). There are usually dense granules in the monocytes and lymphocytes, and often a coarsening of the already heavy granules in the basophils (Plate II, Dg). A similar change in the mast cells of the marrow is claimed to be a sign of diagnostic value (Undritz, 1958b). In the complete syndrome other tissues are affected by the accumulation of storage material, and characteristic granules have been described in the endothelial cells (Undritz, 1958b).

The nature of the stored polysaccharide seems to vary from site to site. The abnormal granules in the neutrophils have been shown by cytochemical techniques to contain a protein–polysaccharide complex (Astaldi & Strosselli, 1958; Mauri & Soldati, 1958); the material present in the tissues is probably a mucopolysaccharide and that in the nerve cells a ganglioside (Ullrich & Wiedemann, 1953). The changes, however, may be due to a single enzyme defect, with the ultimate expression dependent upon factors in the cells concerned.

Incomplete forms in which only one variety of leucocyte was affected have been reported (see Table I) (Gasser, 1950; Eichenberger, 1954; Grgić & Kalafatić, 1958), and the cases described by Mittwoch (1959) may belong to the Gasser-Zellweger type. In addition, a form in which only some of the neutrophils contain abnormal granules has been described (Alder, 1939; Undritz, 1954).

The main difficulty in diagnosing this anomaly is to distinguish it from toxic granulation (Plate II, Db, Dd), but this latter is temporary, while the Alder-Reilly granules persist throughout life. Also there are certain staining differences—as Astaldi & Strosselli (1958) have pointed out.

Chediak-Steinbrinck Anomaly of Giant Granulations in the Leucocytes

This anomaly of the giant leucocyte granulations is associated with a progressive impairment of the resistance to infection, terminating in death from sepsis during childhood. The anomaly, found in 4 members of a 13 sibship by Béguez César (1943) in 1940, was made known by Chediak (1952). Steinbrinck (1948) discovered the condition quite independently, and cases have been reported from various parts of the world (Hansson *et al.* 1959; Undritz, 1958c).

The anomaly consists of a gross coarsening of the granules in the neutrophils, eosinophils, basophils, lymphocytes and monocytes. The neutrophil granules appear to have clumped together to form masses up to 4.5 μ in diameter, or over ten times the normal size (Undritz, 1958b), and Döhle bodies may be present. It has been suggested that this is evidence of a functional defect, but direct investigation by Saraiva, Azevedo, Correa, Carvalho & Prospero (1959) showed a normal phagocytic activity. Crystals, visible with ordinary magnification, in the eosinophil granules also suggest dysfunction. Inclusion bodies, often recognizable as the remains of granulocytes, are frequently seen in the monocytes. As the disease progresses the monocytes attempt to take the place of the ineffective granulocytes, but eventually the defence system breaks down completely.

No linkage has been shown between the anomaly and skeletal deformities or cerebral changes, but the majority of the affected children were albinos and suffered from photophobia. At one time it was thought that in the non-albino cases there was a better prognosis, but the death of Stein-

brinck's patient (Steinbrinck, 1948) at the age of eight has dispelled this conception (Hansson *et al.* 1959). Most of the children have had hepatosplenomegaly, and a lymphadenopathy in which the architecture of the lymph-nodes was destroyed by an infiltration with lymphocytes and monocytes (Donohue & Bain, 1957; Efrati & Jonas, 1958; Hansson *et al.* 1959; Saraiva *et al.* 1959). As the disorder progressed, anaemia, thrombocytopenia and neutropenia developed and death took place from sepsis resistant to all treatment. Clinically the condition might be confused with acute leukaemia.

Peroxidase Defect in the Neutrophils

Failure of the neutrophil peroxidase activity has been described as an anomaly by Alius (cited without details by Undritz, unpublished).

Hereditary Constitutional Agranulocytosis (Infantile Genetic Agranulocytosis)

This is the best defined of a group of disorders apparently inherited as recessive characteristics. It first manifests itself soon after birth, and death takes place in the neonatal period (Kostmann, 1957). Buckwold & Emson (1959) have reviewed this and the related conditions recorded in the literature. Strictly, agranulocytosis is not an anomaly of the leucocytes but a quantitative change.

* * *

Several of the anomalies described are a part of a generalized metabolic defect and as such provide readily obtainable material for cytochemical and similar studies. Others have provided excellent markers for genetic studies and transplantation experiments.

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INDIRECT ASSESSMENT OF NUMBER OF X CHROMOSOMES IN MAN, USING NUCLEAR SEXING AND COLOUR VISION

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1 Nuclear sexing
  a Applications
  b Relation of sex chromatin to X chromosomes
2 Colour-blindness
  a Group studies
  b Family studies
  c Other sex-linked conditions
References

Several papers in this Bulletin deal with the new techniques of chromosome counting and analysis in man.[1] In the case of the autosomes only, these direct methods can localize a disorder to an individual chromosome. With the sex chromosomes the position is rather different. Except in true hermaphrodites (Ferguson-Smith, Johnston & Weinberg, 1960), a testis is probably invariably associated with the presence of a Y chromosome. In the case of the X chromosomes, very precise deductions can be made, apart from microscopic identification, about the number present, using two independent lines of indirect evidence. The importance of these two lines is fourfold. First, they allowed useful progress to be made in this field before the direct method was available. Second, they offer an independent check of the accuracy of direct methods (still sometimes necessary—see, e.g., Fraccaro & Lindsten, 1960). Third, their technical simplicity makes large surveys possible. Fourth, they sometimes produce results not obtainable in any other way.

The two methods are nuclear sexing and the study of sex-linked characters, of which colour-blindness is undoubtedly the most important. The first method has the wider application, but the two are largely complementary, and they will be dealt with at equal length here, the more so as reviews of nuclear sexing are numerous (e.g., Barr, 1955, 1957, 1961; Lennox, 1956, 1960; Grumbach & Barr, 1958; Hienz, 1959; Barr & Carr, 1960; Lennox, Serr & Ferguson-Smith, 1960), but there are only a few on colour vision (Walls, 1959; Polani, 1961; Stewart, 1961).

1. Nuclear Sexing

Nuclear sexing depends on the recognition in the nucleus of a nodule of chromatin, the sex chromatin, which is absent in the male (Barr & Bertram, 1949). It is recognizable with relative ease in most tissues of man, monkey and cat, in nerve cells of ungulates, and in some other mammals—at least in the polymorph leucocytes—but it is demonstrable only rarely otherwise. In some birds and insects in which the female sex is not of karyotype XX but is XY (though, in view of some uncertainty as to which chromosomes are homologous in man and in other species, the letters Z and W are often used), Barr bodies can be observed in the heterogametic sex.

Several techniques are available for research in man:

i. histological sections; this technique is widely applicable but demands exceptionally good preparations (Moore, Graham & Barr, 1953);
ii. blood films, in which "drumsticks" in polymorphs are counted (Davidson & Smith, 1954; Davidson, 1960);
iii. buccal mucosal smears, the most commonly used method (Moore & Barr, 1955);
iv. other desquamated cells, including those in vaginal smears and amniotic fluid;
v. membrane spreads (Klinger, 1958) and tissue-culture monolayers (Serr, Ferguson-Smith, Lennox & Paul, 1958); these can provide very favourable material.

a. Applications

Applications of these techniques have been numerous (Lennox, 1960), the most important involving sexing of the whole individual. Recognition of the presence of sex chromatin in cases of congenital adrenal virilism and Klinefelter's syndrome (of XXY karyotype, i.e., chromosome complement), and of its absence in cases of testicular feminization and Turner's syndrome (of XO karyotype), have greatly eased the recognition and study of these conditions. Abnormalities of the sex chromatin in some more recently recognized conditions (such as its duplication in individuals of XXX karyotype) are equally valuable. The foetus can be sexed before birth by study of the cells of amniotic fluid (Serr, Sachs & Danon, 1957), and sexing of early abortions offers hope of determining the true sex ratio at conception (Stevenson & McClarin, 1957; Stevenson, 1959; Tricomi, Serr & Solish, 1960). Some points of placental anatomy have been elucidated by its use (Klinger & Ludwig, 1957; Sadowsky, Serr & Kohn, 1957). The behaviour of homografts, whether of skin (Woodruff & Lennox, 1959) or of marrow (Davidson, Fowler & Smith, 1958), can be followed if host and donor are of different sex. Tumours in general follow the sex of the host, with some interesting variations depending on the chromosome anomalies usual in tumours (Atkin, 1960), but with very significant discrepancies in teratomata (Hunter & Lennox, 1954; Lennox, 1961) and in chorio-epitheliomata (Park, 1957).

b. Relation of Sex Chromatin to X Chromosomes

It was originally suggested by Barr & Bertram (1949) that the sex chromatin represented the heterochromatin of the two X chromosomes of the nucleus of the female. Despite some difficulties, this most useful hypothesis was generally accepted (Lennox, 1956). The colour-blindness studies considered below appeared to lend it strong support. Several new lines of evidence have, however, led to its abandonment recently.

i. In subjects with three or more X chromosomes (e.g. XXX, XXXY—see Harnden & Jacobs, 1961), the number of sex chromatin bodies is not half the number of X chromosomes, as it should be on the original hypothesis, but is one less than the number of X's (Stewart, 1960).

[1] See Harnden & Jacobs, p. 206, and Penrose, p. 184 of this number of the Bulletin.—Ed.

ii. Tissue-culture studies on the rat (Ohno, Kaplan & Kinosita, 1958), and on man (Ohno & Makino, 1961) indicate that only one chromosome exhibits conspicuous positive heteropyknosis in prophase in the normal female, and none in the male. It is at least reasonable to identify this chromosome as one of the X's and to relate its appearance to that of the sex chromatin.

iii. Polyploid cells (especially as studied in tumours by Atkin, 1960) afford evidence that the number of autosomes must be considered. A tetraploid male cell has two X chromosomes, but no sex chromatin: a tetraploid female cell has four X's, but only two sex chromatin bodies.

The best available hypothesis suggests that all X chromosomes are "potentially" heteropyknotic and capable of giving rise to a sex chromatin body, but that each set of autosomes contains a factor which suppresses the pyknosis of one X chromosome. The number of sex chromatin bodies thus represents the number of X chromosomes present, minus the number of autosome sets (*Lancet*, 1960; Lennox, 1961). Since under most circumstances one can neglect polyploidy in applying nuclear sexing to individuals (the triploid case of Böök & Santesson, 1960, is a possible exception), it is, in general, safe to apply the simple formula of adding one to the number of sex chromatin bodies to give the number of X chromosomes.

Dr Melvin Grumbach (personal communication) has seen two cases of chromatin-positive Turner's syndrome with 45 chromosomes, and apparently XO chromosomes. No satisfactory explanation of these findings has yet been offered, but they are so exceptional that they can perhaps be neglected for the present in framing any general hypothesis.

2. Colour-Blindness

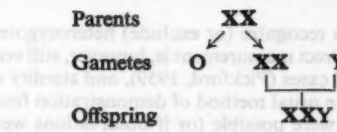
a. Group Studies

Colour-blindness is much the most satisfactory known genetic marker of the X chromosome (Renwick, 1961). The gene has a reasonable frequency, a clear-cut mode of inheritance, and no effect on fertility; if one neglects the existence of several distinct varieties (and for the present purposes, it appears to be safe to do so), its manifestation is unequivocal and fairly easy to determine.

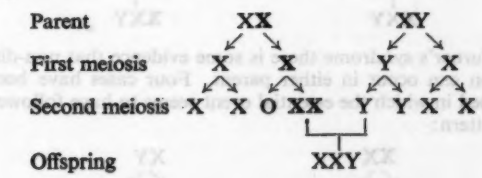
Approximately 8% of normal males (at least in most races, though there are exceptions—Pickford, 1951) are colour blind: it can therefore be assumed that 8% of X chromosomes carry a recessive gene for colour-blindness. A colour-blind female needs the coincidence of two affected X chromosomes: the frequency in females should therefore be 8% of 8%, or 0.64%, though in fact it is nearer 0.4%—the discrepancy deriving probably from the occupation by the several forms of colour-blindness genes of two distinct, though very closely related, loci. The wide difference between the sexes depends only on the difference in the number of X chromosomes, and the difference can be used therefore in abnormal individuals to estimate the number of X chromosomes present. One must, of course, have a group large enough to give a trustworthy estimate of the percentage, and one must be sure also that the group is homogeneous.

Some early attempts to use the method are recorded by Walls (1959). The first success came in connexion with Turner's syndrome. Here, the problem was to prove, without benefit of direct chromosome studies, that the absence of sex chromatin in these apparent females was a genuine reflection of the sex chromosome constitution. Polani, Lessof & Bishop (1956) and Bishop, Lessof & Polani (1960) found four colour-blind persons among 29 chromatin-negative cases—a figure not unlikely to be seen among a similar group of normal males, but extremely unlikely in normal females. Hence it was deduced that they had only one X chromosome; the assumption that they were therefore XY subjects, though natural at the time, was of course unjustified.

The corresponding demonstration that two X chromosomes were present in chromatin-positive Klinefelter's syndrome proved more difficult, larger numbers being needed to demonstrate the lower frequency. Polani, Bishop, Lennox, Ferguson-Smith, Stewart & Prader (1958) collected cases from Glasgow, London and Zürich and found none colour blind among 55 cases (a figure later raised to 73), while there were four in 38 chromatin-negative cases of the syndrome. This correspondence with the figures expected in normal females seemed good evidence for the presence of two X chromosomes. Soon afterwards, however, Nowakowski, Lenz & Parada (1959) found three with colour-blindness among 34 chromatin-positive cases, and considered this equally good evidence for the single X chromosome. If we combine the two series, we get a frequency of about 3%, intermediate between normal male and female frequencies. Now that we know more of the mechanism of those cases, it is possible to explain the discrepancies. Case 5 of Nowakowski *et al.* had a colour-blind mother. Non-disjunction in the ovary, with passage of both X chromosomes into one ovum, could clearly give rise to a colour-blind XXY subject. (In what follows, X represents a normal X chromosome; **X**, one carrying colour-blindness.)



Cases 12 and 19 of Nowakowski *et al.* both had mothers who must have been carriers. Some mechanism other than simple non-disjunction at the reduction division must be involved here: the simplest is non-disjunction at second meiosis.



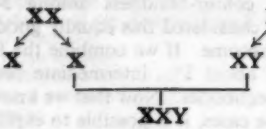
Other more sophisticated explanations are available (Stern, 1959; Stewart, 1961), which lead to the same result. Clearly, in the presence of abnormal gamete formation, the proportion of homozygotes among the offspring will often be greater than expected. This cannot interfere with the findings in single-X cases, but explains the discrepancy in XX cases. With a larger number of X chromosomes (as in XXX and XXXY subjects—see Harnden & Jacobs, 1961), the findings would probably be even less reliable, apart from the unrealistically large series needed to demonstrate, for instance, a predicted frequency of the order of 0.03% (8% of 0.4%) with subjects of XXX karyotype. Findings in individual colour-

blind cases of these syndromes might, however, be exceedingly interesting if supported by family studies of the kind to be described.

b. Family Studies

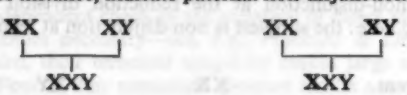
We have already seen examples of the use of these studies in Nowakowski's cases. The first gives excellent evidence of ordinary non-disjunction at first meiosis. The second and third give equally good evidence that some other mechanism can also be involved. In all three, one can deduce that it is maternal gametogenesis which is at fault. Since all three must be XXY subjects, and the father in each, not being colour blind, must be an XY subject, the father must have contributed the Y—and the Y only; therefore the mother must be at fault.

It happens that no similar evidence is available to prove that paternal gametogenesis can be at fault. Confirmation by colour-vision studies of non-disjunction in paternal gametogenesis would require a family of the following type:

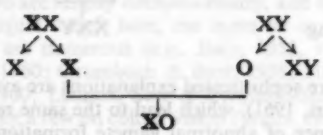


—that is to say, a non-colour-blind subject of XXY karyotype born of a colour-blind mother and a normal father; and this has not been reported. Since, however, such an event would in any case be rare, and since it tends to be only the colour-blind who have their families studied, no great weight need be placed on this.

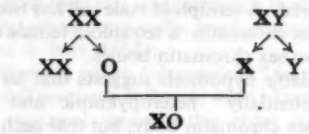
If it were possible to recognize (or exclude) heterozygotes, more could be done. Direct measurement is, however, still very unreliable in individual cases (Pickford, 1959), and sterility of the subjects prevents the usual method of demonstration from family studies. If this were possible (or if observations were possible on some other sex-linked character in which the heterozygote could be recognized), the following events would, for instance, indicate paternal non-disjunction.



In Turner's syndrome there is some evidence that non-disjunction can occur in either parent. Four cases have been described in which the essential event seems to have followed this pattern:



(See Lenz, 1957; Polani, 1961; Stewart, 1961.) Here, the patient cannot have derived either an X chromosome (which would have prevented colour-blindness) or a Y (which would have prevented Turner's syndrome) from her father, and unless she is both parthenogenetic and monosomic (a possibility which Platt & Stratton, 1956, excluded) it must be assumed that paternal non-disjunction has occurred. One case (Frey's, cited by Polani, 1961) indicates probable maternal non-disjunction, thus:



In the case of testicular feminization, no series is available to demonstrate the frequency of colour-blindness. Since these subjects have XY chromosomes, the frequency will almost certainly be that of normal males. Stewart (1959) reported one colour-blind case. His patient had a non-colour-blind affected sib. This proves only that if the locus for testicular feminization lies on the X chromosome, it must lie far enough away for the colour-blindness locus to allow of free crossing-over: it is, however, at least not incompatible with the view that the gene is a sex-limited dominant carried on an autosome. Clearly, in all these conditions further data would be welcome.

c. Other Sex-Linked Conditions

Little use has yet been made of conditions other than colour-blindness as sex chromosome markers. The use, by Polani, Hunter & Lennox (1954), of coarctation of the aorta as a guide to cases of Turner's syndrome especially likely to have an anomalous sex chromosome constitution, might be claimed as an example.

Recognition of the existence of sex chromosome anomalies has eased the explanation of some irregularities of inheritance of sex-linked characters—notably, of course, with colour-blindness itself (Lenz, 1957; Walls, 1959). Some fraction—not altogether negligible—of the incidence of colour-blindness in women may be made up of cases of the Turner's syndrome group and of testicular feminization. At least one female haemophiliac has been shown to be chromatin negative (Nilssen, Bergman, Reitalu & Waldenström, 1959)—a case, one would judge now, most probably of testicular feminization. No such apparent irregularities can now be accepted as proved until chromosomal anomalies have been excluded.

* * *

Despite the new direct techniques, the indirect counting of X chromosomes is still useful. Markers of equal reliability for other chromosomes—especially for chromosome pairs such as numbers 15 and 21 where aberrations occur frequently—would be enormously valuable.

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TURNER'S SYNDROME AND ALLIED CONDITIONS

Clinical Features and Chromosome Abnormalities

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1 History and terminology
2 Syndromes of ovarian dysgenesis
a Clinical features
b Chromosome anomalies
3 Ullrich's syndrome in the female
4 Turner's and Ullrich's syndromes in males
References

This paper refers essentially to clinical disorders in which there is primary maldevelopment of the ovaries, often, but not invariably, associated with multiple somatic malformations.

1. History and Terminology

In 1938, Turner described a syndrome of primary amenorrhoea, webbing of the neck, cubitus valgus and short stature. In 1930, Ullrich had described similar somatic anomalies in a girl and collected from the literature several cases in both males and females, but lack of pubertal development was not observed. While Turner had thought that the osseous and sexual infantilisms were similar to those of pituitary origin, Sharpey-Schafer (1941) thought that the ovaries were primarily defective. Earlier observations by pathologists had already established the association between ovarian maldevelopment and somatic and sexual infantilism (Olivet, 1923; Rössle & Wallart, 1930), but the frequent presence of multiple somatic anomalies had not received special comment. Sharpey-Schafer's suggestion about the origin of the endocrine defect was confirmed by Varney, Kenyon & Koch (1942) and by Albright, Smith & Fraser (1942) who found that the urinary excretion of gonadotropins was increased. The last-mentioned authors stressed the frequent association of somatic anomalies (notably webbing of the neck and coarctation of the aorta), and obtained laparoscopic evidence of ovarian maldevelopment. Albright *et al.* (1942) and Varney *et al.* (1942) alluded to the existence of patients with ovarian agenesis (Olivet, 1923; Wilkins & Fleischmann, 1944) and normal stature, or even tall subjects with eunuchoid body proportions (Pelà, 1935).

For some time considerable speculation existed about the origin of the condition. In 1954 it was found that many patients with ovarian agenesis were chromatin negative on nuclear sexing (Polani, Hunter & Lennox, 1954; Wilkins, Grumbach & Van Wyk, 1954), a fact which was at first interpreted to represent sex-reversal in a chromosomally male embryo (in keeping with the experiments of Jost, 1947). Later, it was suggested that the findings of colour-blindness and of nuclear sexing in ovarian agenesis were compatible with the presence of an XO sex chromosome anomaly in some patients (Polani, Lessof & Bishop, 1956) and even with mosaicism in others, when special nuclear sex scoring techniques were used (Danon & Sachs, 1957). Recent findings have confirmed both these suggestions (Ford, Jones, Polani, de Almeida & Briggs, 1959; Ford, 1961).

Ullrich expanded his clinical description and related his findings to those of Bonnevie in the *my* mice (Ullrich, 1937, 1944, 1949). It became clear that occasionally women were found with some of the somatic stigmata of Turner's syndrome, but with normally functioning ovaries. Webbing of the neck and other anomalies were described in males also and were sometimes found to be associated with malformed or malfunctioning testes (Flavell, 1943).

The clinical terminology and classification of these disorders have become somewhat confusing. "Turner's syndrome" is used by some synonymously with "ovarian agenesis", whereas others use the eponym "Bonnevie-Ullrich's syndrome". Ovarian agenesis has been renamed "gonadal dysgenesis", but sometimes this term is used in a wider, nonspecific sense to imply any error of gonadal development. Yet other names[1] have been used.

It is difficult to devise a clinical terminology which is accurate and descriptive—yet to do so is important, particularly at the present time when the chromosome findings need careful correlation with the clinical features.

The following terminology and clinical classification will be used throughout this paper.

In Females

a. Ovarian (gonadal) dysgenesis
   i. with webbing of the neck (and often other anomalies) and short stature (Turner's syndrome in the female);
   ii. without webbing of the neck, but with small stature;
   iii. pure gonadal (ovarian) dysgenesis without obvious congenital anomalies and with normal stature;
   iv. gonadal dysgenesis with male pseudo-hermaphroditism.

b. A condition similar in appearance to ovarian (gonadal) dysgenesis with webbing of the neck, but with normal ovaries and normal sex functions after puberty (Ullrich's syndrome in the female).

In Males

In line with the terminology used in females:

i. webbing of the neck and other anomalies; testicular abnormality of size, structure or function (Turner's syndrome in the male);
ii. webbing of the neck and other anomalies; sex organs and functions normal (Ullrich's syndrome in the male).

2. Syndromes of Ovarian Dysgenesis

Many women with ovarian dysgenesis are chromatin negative on nuclear sexing. Of 117 patients, 98 were chromatin negative and 16 chromatin positive (Segal & Nelson, 1957). The proportion of chromatin-negative subjects is greatest in

[1] Turner's syndrome, ovarian agenesis, ovarian dysgenesis, gonadal dysgenesis, gonadal dysplasia, gonadal aplasia, status Bonnevie-Ullrich, Ullrich's syndrome, germ cell aplasia, pseudo-females, sexual infantilism, ovarian infantilism, syndrome of rudimentary gonads with dwarfism, pterygonuchal infantilism.

the group with webbing of the neck and lowest in pure gonadal dysgenesis.

In assessing the nuclear sex status, attention is paid not only to the frequency of nuclei with sex chromatin masses, but also to the number of the latter per nucleus and to their size. All these factors are considered important in the interpretation of the sex chromosome findings.

a. Clinical Features

These have been considered in some detail in various surveys (Lisser, Curtis, Escamilla & Goldberg, 1947; Grumbach, Van Wyk & Wilkins, 1955; Hoffenberg & Jackson, 1957a, 1957b; Grumbach & Barr, 1958; Haddad & Wilkins, 1959; Bishop, Lessof & Polani, 1960; Hauser, 1961).

i. Sexual Infantilism

This becomes evident at the time of expected puberty, when primary amenorrhoea is a striking feature of all variants of ovarian dysgenesis. It should be stressed, however, that occasionally a history is elicited of a few spontaneous menstrual periods or, exceptionally, of more regular menstruation (Hoffenberg, Jackson & Muller, 1957).

In the absence of any treatment the breasts are generally undeveloped, but perhaps one case of every five might show slight or even more marked development (Hoffenberg & Jackson, 1957a). The areolae are also grossly hypoplastic, a fact which may be obvious already in childhood, and the nipples are often widely spaced, particularly in those subjects who have a "shield" or a "funnel" chest deformity.

Usually sexual hair is scanty; its complete absence is exceptional. Often the axillary hair is implanted along the inner surface of the arm rather than high in the axillary fossa. It is interesting that in normal males the axillary hair has a more distal site of growth than in females (Epstein, 1932).

The external genitalia are infantile: the labia majora are neither pigmented nor prominent and the labia minora may be unapparent; the clitoris is usually normal, but is enlarged in some patients (variant of gonadal dysgenesis with phallic enlargement—Meyer, 1925; Pich, 1936–37; Gordan, Overstreet, Traut & Winch, 1955; Greenblatt, Carmona & Higdon, 1956).

The uterus is infantile, the tubes narrow and long. The gonads are represented by long, narrow, white streaks situated in the position normal for the ovary. They are composed of connective tissue often arranged in whorls, while, deep in the hilus, structures usually interpreted as rete ovarii are often seen. Clumps of large, clear "hilus cells" are sometimes present, particularly in the patients with phallic enlargement. Ovarian follicles are absent but whether the absence is complete or not, even in infancy, is not known (Blanc & Grumbach, 1960). It should be remembered that a clinical diagnosis of ovarian dysgenesis is often made without confirmation by laparotomy.

Studies of hormone excretion after puberty show that the output of 17-ketosteroids is lower than normal (for women) in about half the cases. The output of gonadatropins is often markedly increased. However, in about one-quarter of cases normal values are obtained (Bishop *et al.* 1960). In part, this may be because the biological assay of urinary gonadotropins is erratic, and not much reliance can be put on a single estimation.

ii. Stature, Birth-Weight and Skeletal Abnormalities

Patients with ovarian dysgenesis, with and without webbing of the neck, are invariably short but, by definition, this is not so in pure gonadal dysgenesis. (Some might prefer to restrict this name to patients with eunuchoid body proportion (Swyer, 1955; Greenblatt *et al.* 1956; Hoffenberg & Jackson, 1957b; Harnden & Stewart, 1959)). Shortness of stature may be noticed early in life—height below the third percentile—and may constitute a reason for seeking medical advice even in the absence of other associated anomalies. In adults, the dividing point between abnormal smallness and normality can be arbitrarily fixed at 4 ft.[2] 11 in.[3] The height of most adults with ovarian dysgenesis does not exceed 4 ft. 9 in. and this is particularly true of those with webbing of the neck.

The level of circulating growth hormone appears to be as high as in acromegaly in spite of the short stature (Fraccaro, Gemzell & Lindsten, 1960), a finding which suggests an unresponsiveness of the target organs or tissues. On the other hand, some response to human growth hormone has been detected (Escamilla, Hutchings, Deamer & Li, 1960; Forbes, Jacobsen, Caroll & Pechet, 1960).

The birth-weight of patients with ovarian dysgenesis tends to be low, often within the range of prematurity (5.5 lb.[4] and under). On the whole, it is in the more malformed subjects, i.e., in those with webbing of the neck, that the birth-weight tends to be lowest. There are many exceptions to this rule and in this connexion it is worth remembering the presence of oedema in many new-born babies with ovarian dysgenesis.

Epiphyseal formation appears to be only a little delayed (Haddad & Wilkins, 1959) when carefully compared with existing standards of bone maturation, but skeletal maturation ceases at the time of expected puberty (Acheson & Zampa, 1961), when failure of post-pubertal epiphyseal closure occurs. Epiphyseal closure appears to take place under the influence of treatment with oestrogens. In any assessment of skeletal age it must be remembered that the bones themselves are abnormal in both their coarser and finer structure, and that the whole pattern of skeletal growth greatly departs from normality at any age—so that one is not dealing only with simple retardation.

Skeletal anomalies are often seen: they may involve the face (micrognathia), the spine (changes resembling those seen in Scheuermann's disease, occasional fusion of cervical vertebrae as in the Klippel-Feil syndrome), the chest ("shield" chest (Lisser *et al.* 1947), "funnel" chest), the pelvis (android shape), the limbs (increased carrying angle of the elbow in a proportion of cases), Madelung's deformity of the wrist, and occasionally changes of the medial tibial condyle (Kosowicz, 1959). All these anomalies are more common in patients with webbing of the neck, but changes of the structure of the hands are equally common in the variants with and without webbing; these are obvious in perhaps one in every four patients and may consist of digital anomalies (camptodactyly, syndactyly) or in what has been termed pseudo-pseudo-hypoparathyroidism (brachymetacarpalism). These changes result from premature fusion of the metacarpal epiphyses with consequent shortening of the metacarpals, particularly on the ulnar side (Kosowicz, 1959; van der Werff ten Bosch, 1959; Hortling,

[2] 1 foot = 30.48 cm.—Ed.
[3] 1 inch = 2.54 cm.—Ed.
[4] 1 pound = 0.454 kg.—Ed.

Puupponen & Koski, 1960). However, short metacarpals can be found in women who have normally functioning ovaries (van der Werff ten Bosch, 1959; Hortling *et al.* 1960). Finally, osteoporosis, found in a proportion of patients, is sometimes severe enough to cause vertebral collapse (Hoffenberg & Jackson, 1957a). The rarefaction tends to involve the cervical, lumbar and coccygeal vertebrae, the lower femoral epiphyses and the carpal and tarsal bones (Kosowicz, 1959).

iii. Tegumentary System and Facies

Webbing of the neck is the characteristic sign of one of the two major variants of ovarian dysgenesis. Although it is usually well evident, there are borderline cases when its existence may be in doubt, the more so because in some patients the neck may be unduly short and wide without being webbed. When present at any age, it draws attention to the possibility that ovarian dysgenesis may exist and this is often, though not invariably, the case (see section 3). In new-born babies webbing of the neck may be seen, but it is more common to find a redundancy of the skin of the back of the neck, which gradually disappears as webbing becomes evident. Webbing is found in about half the cases in most published hospital series (Haddad & Wilkins, 1959; Bishop *et al.* 1960; Jacobs, Harnden, Buckton, Court Brown, King, McBride, MacGregor & Maclean, 1961), though in some series it has been much less prevalent (Albright *et al.* 1942; Hoffenberg & Jackson, 1957a). Webbing of the fingers or of the axillae is seen occasionally.

Some patients have a peculiar facies, particularly those with webbing of the neck. This is due to a variety of changes, single or combined: the smallness of the mandible, the anti-mongolian slant of the eyes, the depressed corners of the mouth, the low-set ears, the presence of pigmented naevi and occasionally the associated ptosis (which gives the patients an expression like that seen in Moebius' syndrome). The auricles are at times malformed as well as displaced and projecting, a fact which in some instances may point to the presence of renal anomalies (Bishop *et al.* 1960). At times the type of auricular malformation is like that described by Hilson (1957) in association with renal abnormalities.

The presence of pigmented naevi was commented on by Turner (1938), and undoubtedly some patients, not necessarily only those with webbing, have an excessive number of them of various sizes, some flat and some raised. Zampa (personal communication), by making mole counts in patients and normal controls matched for sex, age, and eye and hair colour, found a significant excess of naevi in patients with ovarian dysgenesis. Other pigmentary changes are seen, such as vitiligo or café-au-lait spots. The association has been noticed with von Recklinghausen's neurofibromatosis (probably Pich, 1936–37, case II; probably Grumbach *et al.* 1955; Bishop *et al.* 1960).

The nails are often malformed: in young infants they may be very small, while in older subjects they often appear set deep into the nail bed.

Congenital lymphoedema of the legs, feet and hands is present in about one-quarter of all patients (Bishop *et al.* 1960), though it tends to disappear after a few months or years. It is more common in patients with webbing of the neck. Oedema of the legs sometimes appears in later life when treatment with oestrogens or anabolic steroids is undertaken.

After surgical operations there is a tendency in some patients to keloid formation, a fact which should be remembered when plastic correction of webs is undertaken (Haddad & Wilkins, 1959). In some patients the skin of the face is wizened and gives an impression of senility.

In patients with ovarian dysgenesis considered as a group, anomalies of the pattern formed by the palmar dermal ridges are seen (Penrose and Polani, unpublished). These consist essentially of a distal displacement of the distal palmar triradius (less marked on an average than in mongolism).

iv. Visceral Anomalies

The most important are the cardiovascular changes of which coarctation of the aorta is the most common. In the general population it would appear that coarctation of the aorta is more frequent in males. In patients with ovarian dysgenesis and webbing of the neck who are chromatin negative, if congenital heart disease is present this appears to be invariably coarctation of the aorta; however, coarctation has been seen in Ullrich's syndrome in the female (Bishop *et al.* 1960). In some hospital series the association between coarctation and chromatin-negative ovarian dysgenesis with webbing of the neck was found in between one-third and one-quarter of the patients (Haddad & Wilkins, 1959; Bishop *et al.* 1960). In other series, the frequency was considerably lower (Albright *et al.* 1942; Lisser *et al.* 1947; Hoffenberg & Jackson, 1957a). Other types of congenital heart disease are less frequent (Haddad & Wilkins, 1959); we have not observed them in any patient with ovarian dysgenesis, although we have seen them frequently in subjects with a webbed neck but with normal ovarian function (Bishop *et al.* 1960).

In a proportion of patients without evidence of aortic coarctation, a slight degree of diastolic and systolic hypertension is found, generally somewhat fluctuating, but apparently not progressive. It is equally frequent in patients with and without webbing of the neck (Haddad & Wilkins, 1959).

Renal anomalies (Meyer, 1925) are often found, though they are usually asymptomatic (Hortling, 1955). Grosser anomalies (horseshoe kidney, double ureters) may have a frequency of about one in every four patients, and minor rotational malformations are much more common. In our experience, horseshoe kidney was the commonest malformation and was found in chromatin-negative subjects, generally with a webbed neck (Bishop *et al.* 1960). In the general population, horseshoe kidney appears to be commoner in males than in females, and the converse appears to be true for ureteric duplication (Thompson, 1929).

Severe gastrointestinal haemorrhage from intestinal haemangiomata, mentioned by Lisser *et al.* (1947), was found in four of their series of 55 patients by Haddad & Wilkins (1959).

v. Intellectual Impairment and Other Anomalies

Slight intellectual impairment is found in some patients, particularly those with webbing of the neck (Haddad & Wilkins, 1959; Polani, 1960). In contrast to chromatin-positive Klinefelter's syndrome (Ferguson-Smith, 1958) and to cases of triplo-X in females (Fraser, Campbell, MacGillivray, Boyd & Lennox, 1960), the condition does not appear to be concentrated among the severely intellectually subnormal in institutions, although Jagiello found one

chromatin-positive female with this condition among 196 non-mongol female defectives (Hamerton, Jagiello and Kirman, unpublished, 1961). Other anomalies are occasionally reported, such as pes cavus, cleft palate, a high arched palate, often among the chromatin-negative subjects. Major red-green colour-vision defects among the latter are as frequent as among normal males (Polani *et al.* 1956).

b. Chromosome Anomalies

i. In Ovarian Dysgenesis with and without Webbing of the Neck

A chromosome number of 45 and an XO sex chromosome constitution have been reported in 33 chromatin-negative patients (Ford *et al.* 1959; Jacobs & Keay, 1959; Stewart, 1959, 1960; Tjio, Puck & Robinson, 1959; Court Brown, Jacobs & Doll, 1960; Fraccaro, Gemzell & Lindsten, 1960; Fraccaro, Kaijser & Lindsten, 1960a, 1960b; Harnden, 1960; Sandberg, Koepf, Crosswhite & Hauschka, 1960; Jacobs *et al.* 1961), but many more have been studied. In interpreting the presence of a single X chromosome, one is guided by the fact that these patients have only 45 chromosomes (with only 15 in the 6/12/X group), that they are chromatin negative, that they have only four small acrocentric chromosomes, that they have a sex anomaly (suggesting involvement of sex chromosomes rather than autosomes) and, most important, that they frequently have major red-green colour-vision defects.

Mosaicism

Evidence is accumulating, suggesting that in some chromatin-negative subjects, as in a proportion of chromatin-positive subjects, sex chromosome mosaicism may exist. By chromosome mosaicism is meant the presence side by side, in some or all the tissues of an individual, of cells with two or more differing chromosome make-ups. While mosaicism may be "proliferative" in origin, derived from chromosomally normal or abnormal cells in the course of cell division after the completion of embryogenesis, we are mainly interested here in chromosome alterations arising during mitosis in the course of early cleavage of the zygote ("developmental" mosaicism, Ford, 1961).

The biological interpretation is relatively simple: in the course of somatic division, a mother cell produces two daughter cells with a different chromosome make-up by a process of non-disjunction of the replicated chromosomes or through anaphase lagging and chromosome loss. In the one event, cells will arise respectively trisomic and monosomic for one or more chromosomes, while in the other monosomic and normal cells will be formed. The different chromosomal types divide independently to produce a mosaic individual. The original zygote itself may be either normal or abnormal and several regular cell divisions may take place before the mitotic mishap. All this, and the possible preferential growth abilities of some cell types as compared with others, may explain the variety of mosaics which may be formed and the variation of the proportion of cells of which they appear to be made up.

By contrast, the demonstration of chromosome mosaicism is complex. Firstly, it is possible that not all tissues may be chromosome mosaics. Secondly, to demonstrate mosaicism in a tissue in which it actually exists, the different cell types must be able to proliferate in culture. Mosaicism is suggested when two or more consistent and discrete cell types are found in short-term or long-term tissue culture. This will result in bimodal or possibly plurimodal distributions of chromosome counts in cells; careful analysis of cells with different modes must show chromosome consistency within each mode; the findings must be repeatable in the same tissues of the presumptive mosaic patient (they need not extend to all the tissues sampled). Even when these conditions are fulfilled, the existence of chromosome mosaicism in vivo is not fully proved, though the inference that it exists is strong.

The existence of the following types of sex chromosome mosaicism has been suggested in the chromatin-negative patients with ovarian dysgenesis, with and without webbing of the neck: XO/XX, XO/XY, XO/XYY (Fraccaro, Gemzell & Lindsten, 1960; Sandberg *et al.* 1960; Jacobs *et al.* 1961; Hamerton and Polani, unpublished).

Three classes of chromosome findings in chromatin-positive subjects have been described: sex chromosome mosaicism (mosaic group), structural changes of the chromosome of special morphological type (isochromosome group) and other structural anomalies (fragmented X chromosome group). Another distinctive group may exist (see section 4).

None of the patients in the mosaic group had webbing of the neck, a fact which may be of interest in relation to the proportion of normal XX cells which these patients are presumed to possess. In five patients it was suggested that there was chromosome mosaicism with cells with 46 chromosomes (XX) and with 45 (XO) (Ford, 1961; Jacobs *et al.* 1961). In one patient an XO/XXX constitution was suggested (Jacobs *et al.* 1961), with cells with 45 and 47 chromosomes, and in a further patient an XO/XX/XXX sex complement of three types of cells is probable (Hamerton and Polani, unpublished).

Four patients presenting with a clinical picture of ovarian dysgenesis have now been reported with another variant of sex chromosome constitution. Their cells have 46 chromosomes (Fraccaro, Ikkos, Lindsten, Luft & Kaijser, 1960; Jacobs *et al.* 1961). Hamerton, Jagiello, Kirman and Polani (unpublished) have seen three more such patients. On analysis there appears to be only one X chromosome but three resembling the third chromosome. It was thought therefore that one of these might represent a structurally altered X chromosome. The most plausible explanations seem to be either that the extra chromosome resembling a no. 3 was an X chromosome, to which a fragment of another chromosome was attached, or that it was isochromosomic, being formed by two long arms of an X.

Isochromosomes derive from a misdivision of the centromere which, instead of splitting longitudinally, as is usual, splits transversely. This results in the formation of two symmetrical chromosomes, each of which is made up to two genetically similar segments. Two isochromosomes could arise from an X chromosome: one would consist of the two genetically similar long arms and the other of the two short arms.

The isochromosome hypothesis has in its favour the fact that this type of chromatin-positive ovarian dysgenesis appears to be not uncommon, suggesting a consistent rather than a chance origin. If this explanation is correct, the subjects have three long arms of the X chromosome (two of which are genetically similar) and only one short arm.

A chromatin-positive female—apparently with ovarian dysgenesis without webbing of the neck—has been described whose mucosa cells contained a proportion of unusually small sex chromatin masses (Jacobs *et al.* 1961) and whose cells had 46 chromosomes. The karyotype, in conjunction with the other findings, was interpreted as showing a single normal X and a fragmented X chromosome consisting of the long arm of the X (deletion of the short arm).

ii. In Pure Gonadal Dysgenesis

In one chromatin-negative patient (Harnden & Stewart, 1959) and in another possibly similar case (Netter, Lambert, Lumbroso, Trévoux, Delzant, de Grouchy & Lamy, 1960) with this variant of ovarian dysgenesis, an XY formula has been found. In two, or more, chromatin-positive subjects a normal XX sex chromosome complement has been found (Jacobs *et al.* 1961). The patient described by Jacobs, Harnden, Court Brown, Goldstein, Close, MacGregor, Maclean & Strong (1960) may be placed in this group on clinical grounds. She had ovarian dysgenesis, was of average height and was chromatin positive but with a low proportion of small chromatin masses in the oral mucosa nuclei, and with 46 chromosomes. On these clinical and nuclear sexing grounds she was interpreted as having a normal X chromosome and a fragment of an X chromosome comprising its short arm.

iii. In Gonadal Dysgenesis with Male Pseudo-Hermaphroditism

Bloise, de Assis, Bottura & Ferrari (1960) described a patient with testicular tissue in one gonad; the other gonad consisted of a "streak". The sex chromosome constitution in the bone-marrow was XO. The possibility of gonosomal mosaicism is not excluded in this case. It is possible that the patient, aged four-and-a-half months, described by Hirschhorn, Decker & Cooper (1960), may belong to this clinical group. Biopsy of one gonad was done. After careful search, only two ova were found and some testicular tissue was present in the cortex. In this patient there was evidence of XO/XY mosaicism.

iv. Other Findings; Frequency

In one chromatin-positive patient with ovarian dysgenesis without webbing of the neck, an XO sex chromosome constitution was found in cells with 45 chromosomes (Grumbach, Morishima & Chu, 1960). A similar finding in another patient coincided with the presence of very small sex chromatin masses (Jacobs *et al.* 1961). There was no evidence of mosaicism in either patient. These findings are interesting in connexion with the work of Ohno and his collaborators on the origin of the sex chromatin (Ohno & Hauschka, 1960), and such subjects may form a distinct chromosome group among the chromatin-positive patients (XO group).

A fertile XO chromatin-negative female of normal phenotype has been described (Bahner, Schwarz, Harnden, Jacobs, Hienz & Walter, 1960). Again, no evidence of somatic mosaicism was found. The finding raises the problem of the frequency of such women undetected in the general population. A preliminary estimate of the prevalence of the XO condition is one in approximately 5,000 women. It is conceivable that the prevalence of the condition varies in different age-groups because of the potential lethality of some of the associated anomalies.

3. Ullrich's Syndrome in the Female

The general clinical features of this condition are similar to those seen in ovarian dysgenesis with webbing of the neck, except that secondary sex development after puberty is normal and normal ovaries are found, or are reasonably assumed to be present. It would appear that coarctation of the aorta is seldom found, though other congenital heart disease is frequent (Bishop *et al.* 1960). The patients are chromatin positive. Before puberty, indirect differentiation from chromatin-positive ovarian dysgenesis with webbing of the neck is probably impossible.

In one patient an autosomal anomaly has been found and in another no anomaly existed (Hamerton and Polani, unpublished). On the grounds of the presence of webbing of the neck and multiple anomalies, the patient described by Edwards, Harnden, Cameron, Crosse & Wolff (1960) may be classified in this group. In this patient a chromosome number of 47 was found, with trisomy of chromosome 17. More examples of this condition have been seen.

4. Turner's and Ullrich's Syndromes in Males

Webbing of the neck and other of the anomalies described above are occasionally seen in males. While genital and testicular development and function may be normal ("Ullrich's syndrome in the male"), these are not always so. The testes may appear small, especially after puberty, and their function abnormal; or testicular descent may not have occurred; or the genital tract may be malformed (hypospadias), suggesting intra-uterine impairment of testis function. In all these circumstances, the microscopical architecture of the testis is probably disturbed, particularly after puberty. The term "Turner's syndrome in the male" has been used to describe the association between testicular and somatic changes (Flavell, 1943). Fifteen cases conforming to the above criteria have been described in the literature (Matolcsy, 1936; Bizarro, 1938; Flavell, 1943; Dorff, Appelman & Liveson, 1948; Greenblatt & Nieburgs, 1948; de Ulhôa Cintra, Mattar, de Almeida & de Oliveria, 1950; Caflisch, 1952, cases 194 and 197; Mackenzie, 1953; Halonen, Seppälä & Hakkila, 1956; Jackson & Hoffenberg, 1957, cases 2 and 3; de la Balze, Aslan & Gurtman, 1958; Becker, 1958; Grumbach & Barr, 1958; Fraccaro, Ikkos, Lindsten, Luft & Tillinger, 1961). Other possible cases exist (McCullagh, 1948; Prunty, McSwiney & Clayton, 1953; Bishop *et al.* 1960; van der Werff ten Bosch, 1960). Testicular biopsies were made in only some of these patients. Congenital heart disease appears to be often associated with the other somatic anomalies (Bishop *et al.* 1960). A normal chromosome complement has been found in the few cases studied (Court Brown *et al.* 1960; Fraccaro *et al.* 1961; Hamerton and Polani, unpublished).

Perhaps the severely intellectually subnormal boy with webbing of the neck and small testes described by Ferguson-Smith, Johnston & Handmaker (1960) could be included in this group of patients. However, unlike other males with Turner's syndrome, he was tall (170.5 cm.). He had a chromosome number of 48 with an XXXY sex formula, and was chromatin positive, many of the mucosa cell nuclei containing two sex chromatin masses.

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CYTOGENETICS OF ABNORMAL SEXUAL DEVELOPMENT IN MAN

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It is now known that many aberrations of sexual development in man are associated with abnormalities of the sex chromosomes. Normally man has 22 pairs of autosomes and one pair of sex chromosomes. In the female the two sex chromosomes are identical and are called X chromosomes, and in the male there is one X chromosome and one Y chromosome. The X chromosomes are medium sized and sub-metacentric and, at present, cannot be differentiated from the autosomes in the same size range, while the Y chromosome is small and acrocentric, similar to autosomes nos. 21 and 22. It can usually be distinguished from these because its centromere is more nearly terminal and because the long arms tend to lie close together, giving it a quite characteristic appearance.

1. Diagnosis of Sex Chromosome Abnormalities

Since the X chromosome cannot be identified unequivocally, great care must be exercised in the interpretation of results of chromosome analyses of cells from any individual suspected of having an abnormality of the chromosomes.

In considering the karyotype of any patient, and particularly those with abnormalities of sexual development, three factors must be considered before a conclusion is reached. These are:

i. chromosome counts and analysis;
ii. sex chromatin and "drumstick" studies;
iii. the clinical condition of the patient.

Study of the sex chromatin is of fundamental importance in this connexion. In normal females a single strongly Feulgen-positive body is found applied to the inside of the nuclear membrane of a proportion of interphase cells from a variety of different tissues (Barr & Bertram, 1949). For convenience, cells of the buccal mucosa are most frequently used for this examination, and in our laboratory between 36% and 51% of these cells from a normal female have a sex chromatin body. In contrast, this body is never found in normal males. Individuals having a sex chromatin body are said to be chromatin positive, while those without are chromatin negative. The present evidence suggests that variations outwith the normal range, both in the percentage of cells with a sex chromatin body and in the size of this body, are significant.

There is also a morphological difference between the polymorphonuclear leucocytes of the female and those of the male. In the normal female the nuclei of a small proportion (1–5%) of these cells bear characteristic club-shaped projections usually termed "drumsticks" (Davidson & Robertson Smith, 1954). These are never found in normal males.

Thus, before a final conclusion is reached all the appropriate information must be considered, and this will be made clearer by giving an example. If it is known only that a male patient has 47 chromosomes, the extra one being in the group of chromosomes similar to the X chromosome, the evidence is insufficient to reach a conclusion on the nature of the extra chromosome. If, however, it is also known that the patient is chromatin positive, has drumsticks on the polymorphonuclear leucocytes and has small atrophic testes, the information is now adequate to justify the conclusion that the extra chromosome is an X and therefore that the patient has an XXY sex chromosome constitution.

2. Discussion of Cytological and Clinical Data

The evidence which has accumulated so far indicates that abnormalities of the sex chromosomes are relatively common, while abnormalities of the autosomes, with the exception of the one associated with mongolism, are much less frequently encountered. Tables I, II, III and IV summarize the cytogenetic and clinical findings in cases of abnormal sexual development in man.

Since there is considerable confusion in the terminology of the various clinical entities which are involved (and indeed many patients do not fit into any clearly defined clinical category), the cases have been grouped in the tables on the basis of the phenotypic sex and the result of sex chromatin and chromosome examinations. It must be pointed out that some of the categories in the tables are based on a small number of cases, and when more have been examined the features listed may not prove to be typical.

a. Phenotypic Males

It is remarkable that six of the seven categories of phenotypic males with an abnormal chromosome constitution (Table I) can all be classified under the general term "Klinefelter's syndrome". The first four groups are, at present, clinically indistinguishable, but there is some evidence to suggest that in the remaining three categories where more than two X chromosomes are present the disturbance of both sexual differentiation and mental development are more profound. Indeed, the four persons with an XXXY sex chromosome constitution and the one who is an XXXY/XXXXY mosaic subject, could all be classified as having

TABLE I. Phenotypic Males with Sex Chromosome Abnormalities

Category no.	Sex chromatin	Drumsticks	Chromosome no.	Sex chromosomes	Clinical features	References
1	Positive	Present	47	XXY	Small atrophic testes with hyalinization of the seminiferous tubules often associated with gynaecomastia; poorly developed secondary sex characters; eunuchoid proportions and mental retardation (Klinefelter's syndrome)	Jacobs & Strong (1959); Harnden (1960); Nowakowski, Lenz, Bergman & Reitalu (1960)
2			46/47	XX/XXY		Ford, Polani, Briggs & Bishop (1959); Hayward (1960)
3			46/47	XY/XXY		Buckton, Tough, Jacobs, Baikie, Court Brown, Maclean and Methven (unpublished, 1961)
4			48	XXYY		Barr & Carr (1960); Muldal & Ockey (1960)
5	Two sex chromatin bodies in a proportion of cells	Present—occasional cell with two drumsticks	48	XXXY	Similar to above, but with more severe testicular atrophy and mental retardation	Barr & Carr (1960); Ferguson-Smith, Johnston & Handmaker (1960)
6	Three sex chromatin bodies in a proportion of cells	Present	48/49	XXXY/XXXXY	Tiny ovoid structures just palpable in the scrotum; poorly developed secondary sex characters; marked eunuchoid proportions; prognathism and severe mental retardation	Buckton *et al.* (unpublished, 1961) (1 case)
7		?	49	XXXXY	Very small testis devoid of structure in right scrotum; malformed external genitalia; peculiar facies; congenital heart lesion and mental retardation	Fraccaro, Kaijser & Lindsten (1960); Fraccaro & Lindsten (1960) (1 case)

severe forms of Klinefelter's syndrome, while the XXXXY subject described by Fraccaro, Kaijser & Lindsten (1960) is even more severely affected, the external genitalia being grossly malformed.

b. Phenotypic Females

In Table II, seven of the twelve categories of females with abnormalities of the sex chromosomes have all cells, or a proportion of cells, with an XO sex chromosome constitution. All these persons are of short stature and the majority have streak gonads, primary amenorrhoea and one or more of the range of congenital anomalies which are usually associated with Turner's syndrome. It is remarkable that all four types of mosaic subjects should show so many of the features which are found in those who have only XO cells. It is possible, however, that these cases were selected because they did show these features, and it is likely that in cases of mosaicism the phenotype will depend on the distribution of the different stem lines in the body. For example, the XO/XY mosaic constitution has been found in true hermaphrodites as well as in women with primary amenorrhoea. It may be that this variability is due to the sex chromosome constitution of the cells of the primitive gonads. Indeed, it seems likely that the subject reported by Bloise, de Assis, Bottura & Ferrari (1960) as an XO male is, in fact, an XO/XY mosaic in whom at least one gonad was composed largely of XY cells (Hirschhorn, Cooper & Miller, 1960).

It is probable that the females with one exceptionally large X chromosome (category 9, Table II) have an isochromosome for the long arms of one of the X chromosomes, since it is unlikely that an exactly similar duplication giving rise to a metacentric chromosome would occur in a number of different cases. If this is true these patients are monosomic for the short arm of the X and trisomic for the long arm. It is interesting to note the similarity between these cases and the one (category 6, Table II) which had a simple deletion of most of the short arms of one X. These patients can, like those with XO cells, be classified as having Turner's syndrome. In contrast, the patient who had a deletion of the long arms of the X while she had primary amenorrhoea was not of unusually short stature and therefore did have the most constant feature of Turner's syndrome.

Females with an XXX or XXXX sex chromosome constitution have no consistent phenotypic abnormalities, in contrast to those with XO cells. Almost all such females who have so far been described have been mental defectives, but as they have been found during routine surveys in institutions for the mentally defective, it is as yet too early to say whether additional X chromosomes are necessarily associated with mental retardation.

c. True Hermaphrodites

It seems very reasonable that an individual with both XY and XO stem lines should develop both testicular tissue and

TABLE II. Phenotypic Females with Sex Chromosome Abnormalities

Category no.	Sex chromatin	Drumsticks	Chromosome no.	Sex chromosomes	Clinical features	References
1	Negative	Absent	45	XO	Phenotype variable; short stature almost always associated with streak gonads and primary amenorrhoea and often also with neck webbing, cubitus valgus, co-arctation of the aorta and digital anomalies (Turner's syndrome)	Ford, Jones, Polani, de Almeida & Briggs (1959); Bahner *et al.* (1960); Fraccaro, Gemzell & Lindsten (1960)
2			45/46	XO/XY		Blank, Bishop & Caley (1960); Jacobs *et al.* (1961)
3			45/46	XO/XX		Ford (1960); Fraccaro, Gemzell & Lindsten (1960)
4			45/47	XO/XYY		Jacobs *et al.* (1961)
5	Anomalous; very small percentage (7%) of bodies which were smaller than normal	Absent	46	XX (Presumed deletion of part of long arm of one X chromosome)	Streak gonads; primary amenorrhoea; infantile external genitalia; no breast development	Jacobs *et al.* (1960) (1 case)
6	Positive—bodies smaller than normal	Present—? smaller than normal	46	X^ (Presumed deletion of most of short arm of one X chromosome)	Short stature; primary amenorrhoea; infantile external genitalia; no breast development; no pubic or axillary hair Turner's syn.	Jacobs *et al.* (1961) (1 case)
7	Positive	Present	45/46	XO/XX	Phenotype variable; short stature; primary amenorrhoea and occasionally webbing of the neck; cubitus valgus and congenital heart lesions (Turner's syndrome)	Ford (1960); Jacobs *et al.* (1961)
8			45	XO	As above	Grumbach, Morishima & Chu (1960)
9	Positive. Unusually high percentage of bodies, some of which are larger than normal	Present. Unusually frequent; some appeared larger than normal	46	XX (Presumed isochromosome of long arms of one X, or duplication of part of short arms)	Primary amenorrhoea; streak gonads; short stature; infantile external genitalia; no breast development	Fraccaro, Ikkos, Lindsten, Luft & Kaijser (1960); Jacobs *et al.* (1961)
10		Present—occasional cell with two drumsticks	47	XXX	Phenotype variable. Usually normal females who are often mentally retarded; but occasionally may be associated with menstrual disturbances and lack of development of secondary sex characters	Jacobs, Baikie, Court Brown, MacGregor, Maclean & Harnden (1959); Barr & Carr (1960); Fraser *et al.* (1960); Stewart & Sanderson (1960)
11	Two sex chromatin bodies in a proportion of cells	Absent	45/47	XO/XXX	Phenotype variable. Primary amenorrhoea; infantile external genitalia and poorly developed secondary sex characters which may be associated with congenital anomalies such as absent vagina and uterus; abnormal bodily proportions and short stature	Barr & Carr (1960); Jacobs *et al.* (1960); Jacobs *et al.* (1961)
12	Three sex chromatin bodies in a proportion of cells	?	48	XXXX	Phenotypically normal females with mental retardation	Barr & Carr (1960) (2 cases)

TABLE III. True Hermaphrodites

Category no.	Sex chromatin	Drumsticks	Chromosome no.	Sex chromosomes	Clinical features	References
1	Positive	Present	46	XX	Phenotype variable; external genitalia ambiguous; both ovarian and testicular tissue present in the gonads	Harnden & Armstrong (1959) Hungerford, Donnelly, Nowell & Beck (1959) de Assis, Epps & Bottura (1960) Ferguson-Smith, Johnston & Weinberg (1960) Gordon, O'Gorman, Dewhurst & Blank (1960) Sasaki & Makino (1960)
2	Positive	?	46/47	?XX/XXX	As above	Ferguson-Smith, Johnston & Weinberg (1960)
3	Negative	Absent	45/46	XO/XY	As above	Hirschhorn *et al.* (1960)

rudimentary ovarian tissue (Table III). On the other hand, the development of testicular tissue in those cases of true hermaphroditism where only female sex chromosomes have been found, is more difficult to explain. It may be possible that some of these cases are in fact mosaics, perhaps of the XX/XXY type. However, because of the large number of cases which have been described, this seems rather unlikely to be true for all of them, and it would therefore seem that testicular tissue can develop in the absence of a Y chromosome.

d. Abnormal Sexual Differentiation

In the examples shown in Table IV the sex chromosome constitution is normal, although in cases of testicular feminization and of chromatin-negative pure gonadal dysgenesis the sex chromosomes are at variance with the phenotypic sex. In these persons, therefore, sex determination is normal and one must postulate different mechanisms for the abnormal sexual differentiation. These mechanisms may still be genetic but on the level of the gene rather than on that of the chromosome. There is good evidence that testicular feminization may be due to the action either of a sex-linked recessive gene or a sex-limited autosomal dominant gene (Evans & Riley, 1953). Some cases of pure gonadal dysgenesis also appear to be genetically determined, as several instances have been reported where more than one member of a sibship is affected. The affected member may be of the same sex chromatin type (Elliott, Sandler & Rabinowitz, 1959) or of a different type (Schönenberg, Hollstein & Kosenow, 1957).

The other possibility is that some environmental factor affects the course of development at a critical stage after the sex has been normally determined. One example of this is the group of cases of adrenal virilism which are due to androgenic hormones reaching the foetus from the maternal circulation (Grumbach & Ducharme, 1960). Some of the so far unexplained cases could have a similar aetiology.

3. Causation of Errors

a. Cases where all the Cells of the Body have an Abnormal Number of Chromosomes

It is probable that most of these cases, where all cells of the body are similarly affected, are the result of primary non-disjunction occurring during gametogenesis in one or other of the parents. It is, of course, possible for non-disjunction to occur at the first or the second meiotic division or at both, and it could occur in either parent or even in both. All such errors will lead to the formation of gametes, and consequently to individuals, with abnormal numbers of chromosomes. With the finding of a fertile XO female (Bahner, Schwarz, Harnden, Jacobs, Hienz & Walter, 1960) and fertile XXX females (Fraser, Campbell, MacGillivray, Boyd & Lennox, 1960; Stewart & Sanderson, 1960), the possibility of the occurrence of secondary non-disjunction must be seriously considered, although no such case has yet been reported.

Usually, it is not possible to say which specific error has given rise to a particular case. Even if one assumes that the majority of cases are due to the occurrence of non-disjunction during gametogenesis, each error can still arise in a number of different ways. However, where a sex-linked genetic trait, such as red-green colour-blindness, is present in the pedigree it may be possible to tell in which parent non-disjunction has occurred (Lennox, 1961).

b. Mosaicism

An error similar to non-disjunction can occur at a mitotic division at any stage subsequent to fertilization. If the products of such a divisional error are viable and are included in the embryo, this would lead to the production of a mosaic individual whose body is composed of two or more types of cell, each with a different number of chromosomes. If the error occurs at the first division of a normal zygote, a 45/47 chromosome mosaic will be formed, but the same error at a subsequent division would lead to the production of a 45/46/47 chromosome mosaic. The XO/XXX mosaic of Jacobs, Harnden, Court Brown, Goldstein, Close, MacGregor, Maclean & Strong (1960) was probably of the former type, while it is possible that the similar mosaic of Jacobs, Harnden, Buckton, Court Brown, King, McBride, MacGregor & Maclean (1961) was of the latter type, although the presence of the 46 (XX) chromosome cells was not considered definitely proved. It would be possible for one chromosome to escape inclusion in either of the daughter cells; this could lead to the additional possibility of a 45/46 chromosome mosaic (e.g., the XO/XX mosaics of Ford, 1960). The composition of the

TABLE IV. Errors of Sex Differentiation

Category no.	Phenotypic sex	Sex chromatin	Drumsticks	Chromosome no.	Sex chromosomes	Clinical features	References
1	M	Negative	Absent	46	XY	Small, often atrophic testes sometimes associated with gynaecomastia; poorly developed secondary sex characters; eunuchoid proportions. (Chromatin-negative Klinefelter's syndrome)	Court Brown, Jacobs & Doll (1960)
2				46	XY	Hypospadias and small atrophic testes often associated with gynaecomastia and failure of development of the secondary sex characters. (Familial and sporadic)	Strong and Jacobs, unpublished
3	F	Negative	Absent	46	XY	Testes always present; uterus and vagina underdeveloped; breasts normal; scanty or absent pubic and axillary hair. Usually familial. (Testicular feminization)	Jacobs, Baikie, Court Brown, Forrest, Roy, Stewart & Lennox (1959); Tjio, Puck & Robinson (1959)
4				46	XY	Streak gonads; infantile genitalia, poor development of secondary sex characters; tall with eunuchoid proportions, sometimes familial. (Pure gonadal dysgenesis)	Harnden & Stewart (1959); de Grouchy, Cottin, Lamy, Netter, Netter-Lambert, Trévoux & Delzant (1960)
5	F	Positive	Present	46	XX	Pure gonadal dysgenesis—as above	Jacobs *et al.* (1961)
6				46	XX	Primary amenorrhoea; short stature and occasionally webbing of the neck; cubitus valgus; congenital heart lesion. (Chromatin-positive Turner's syndrome)	Court Brown *et al.* (1960); Harnden and Jacobs, unpublished
7				46	XX	Primary amenorrhoea; usually of normal stature. Adequate cause known for primary amenorrhoea, e.g., polycystic ovaries, adrenal virilism, congenital abnormalities of uterus, vagina or Fallopian tubes	Court Brown *et al.* (1960); Jacobs *et al.* (1961)

mosaic will also depend on the chromosomal constitution of the zygote. For example, if a Y chromosome is lost from some of the cells of an individual with an XXY sex chromosome constitution, an XX/XXY mosaic will be formed, whereas the same error occurring in an XY individual will produce an XO/XY mosaic. The degree of admixture of the stem lines will depend on the stage of development at which the error occurs, and it is conceivable that one cell line could be localized in a particular tissue. In one of the XO/XY mosaics of Jacobs *et al.* (1961) 89% of the cells from the peripheral blood had 46 chromosomes and an XY sex chromosome constitution, whereas all of the cells from the skin biopsy culture had 45 chromosomes and an XO sex chromosome constitution. The composition of the zygote, the nature of the error and the stage at which it occurs will also undoubtedly influence the clinical condition of the patient.

c. Morphological Abnormalities of the Chromosomes

It is probable that these errors occur during the prophase of meiosis when breakage and rejoining of the chromosomes is occurring as part of the normal process of meiosis. An erroneous rejoining of broken ends can result in several types of error, among which are:

i. Deletion, where a segment of the chromosome is completely lost, e.g. the deleted X chromosome in the cases of Jacobs *et al.* (1960) and Jacobs *et al.* (1961) (Table II).

ii. Duplication, where one segment of the chromosome is represented twice as a result of a translocation of that segment from the homologous chromosome or chromatid. This is one of the possible explanations for the cases of Fraccaro, Ikkos, Lindsten, Luft & Kaijser (1960) and the one case of Jacobs *et al.* (1961) (Table II).

iii. Translocation, where a segment of a chromosome is transferred to a different site on the same or on a different chromosome. If two chromosomes are involved the transfer of material is usually reciprocal. No translocation involving the sex chromosomes has yet been described but there are several instances of a translocation involving the autosomes (e.g., Lejeune, Turpin & Decourt, 1960).

Small changes, however, of any of these types could occur without their being readily observable when the current techniques are used. In some such cases sex chromatin

analysis may deviate from the normal when there is no detectable abnormality of the chromosomes. This may be the explanation for the case of Jacobs *et al.* (1961) in which 28% of exceptionally small sex chromatin bodies were found, with apparently normal autosomes and only one X chromosome that was indistinguishable from normal. It should be remembered that even a small translocation may lead to gross genetic change.

One other possible morphological abnormality must be mentioned as it is the most probable explanation for the cases of Fraccaro, Ikkos, Lindsten, Luft & Kaijser (1960) and of Jacobs *et al.* (1961). If the centromere of a chromosome splits transversely instead of longitudinally, an isochromosome will be formed. This is therefore a perfectly metacentric chromosome with exactly homologous arms united at the centromere.

4. Incidence of Sex Chromosome Abnormalities

a. General Population

Very little is known of the incidence of sex chromosome abnormalities or even sex chromatin abnormalities in the general population, and the only published report is that of Moore (1959). He studied the sex chromatin from buccal mucosa in 3,715 consecutive live-born infants in a single hospital in Winnipeg, Canada. Of the 1,911 males in his series he found five to be chromatin positive, and of the 1,804 females none was found to show an abnormal sex chromatin pattern. A similar survey is being carried out in Edinburgh (Maclean, unpublished, 1961) and, of the 3,000 male infants examined so far in this series, ten were found to be chromatin positive, while in 3,000 female infants one chromatin-negative baby and four with two sex chromatin bodies have been found. It would appear from these surveys that the incidence of chromatin-positive males is of the order of 1:300 to 1:600 live-born male children. When Moore carried out his survey, the significance of two sex chromatin bodies was not appreciated and it is possible that he overlooked any females with double sex chromatin who were born in his series. However, Maclean's figures suggest that females with an additional X chromosome occur with a frequency of approximately 1:1,000. The evidence tends to suggest that chromatin-negative females are born with a lower frequency. On theoretical grounds, XO individuals should be conceived with at least the same frequency as chromatin-positive males and females with double sex chromatin. Their comparative infrequency may be due to a lowered viability of a gamete carrying no sex chromosome or to the fact that an XO sex chromosome constitution is frequently associated with a severe heart lesion, and this may be responsible for the intra-uterine death of a large proportion of individuals conceived with an XO sex chromosome constitution.

b. Mental Defectives

In contrast with the lack of information available on the incidence of sex chromosome abnormalities in the general population, a large number of surveys have been carried out on various groups of mentally subnormal individuals (Ferguson-Smith, 1958, 1959; Prader, Schneider, Francés & Zŭblin, 1958; Mosier, Scott & Cotter, 1960; Maclean, unpublished, 1961).

All these surveys indicate that between 1% and 3% of males with subnormal intelligence in these populations are chromatin positive. They are found with all grades of mental deficiency, but most are high-grade mental defectives and are often admitted to institutions only after repeated incidents of antisocial behaviour.

There are fewer reports available on the incidence of sex chromosome abnormalities in female mental defectives. Fraser *et al.* (1960) reported four females with double sex chromatin out of a total of 595. Maclean (unpublished, 1961) has studied 1,334 female mental defectives and found nine with double sex chromatin. In neither survey was a chromatin-negative female found. The individuals found by Maclean were all very high-grade mental defectives, in contrast to those reported by Fraser who all showed a very marked impairment of their mental ability.

c. Infertility

The only other groups in which the incidence of sex chromosome abnormalities has been studied are males attending an infertility clinic (Ferguson-Smith, Lennox, Mack & Stewart, 1957) and females attending a gynaecological clinic with a complaint of unexplained primary amenorrhoea (Jacobs *et al.* 1961).

In the former group Ferguson-Smith *et al.* (1957) found that 3% of all males attending the infertility clinic were chromatin positive. When he considered only the cases with severe oligospermy (sperm count of less than 1 million per ml.), he found that as many as 11% were chromatin positive.

Of the 44 women with primary amenorrhoea reported by Jacobs *et al.* (1961), 32 were traced and their chromosomes examined. Of these, 17 had abnormalities of the sex chromosomes. This suggests that at least 39% of women with primary amenorrhoea have chromosome abnormalities. From these two surveys it can be seen that an appreciable proportion of cases of infertility among men, and primary amenorrhoea in women, are associated with abnormalities of the sex chromosomes.

5. General Remarks

It is certain that, in the future, chromosome investigations will play an important part in establishing a differential diagnosis for patients with defects of sexual development. Furthermore, cytogenetic study of these patients is not only of academic interest, but should prove of importance to the clinician in several different ways. Thus, by using sex chromatin screening techniques in conjunction with chromosome analysis, many errors of sexual development, which previously could not be accurately diagnosed—or at least not until after puberty—can now be recognized at birth. However, it is unlikely that one will be able to tell whether or not the early recognition of these abnormalities can be of use in attempts to lessen the disadvantageous effects of the abnormal chromosome constitution, until the subjects now being detected have passed the age of puberty.

Chromosome analysis should be of help in other ways. Large-scale family studies may produce evidence of the nature of the mechanisms which control the production of these abnormalities. Epidemiological studies may yield information on the relationship between chromosome abnormalities, defects of development and other pathological conditions. Indeed it seems probable that, since the techniques for the study of human chromosomes have been so recently evolved, the potentialities of human cytogenetic studies have not yet been fully appreciated.

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